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A TYPICAL ACID-FAST BACILLI*

Nocardia and non-chromogenic types

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An interest in *Nocardia* led us into investigation of rapidly growing acid-fast bacilli isolated from patients by the tb laboratory of Cook County Hospital. All colonies appearing on the cultures were stained by Ziehl-Nielsen and the resulting collection of cultures have some degree of acid-fastness as the only common factor. Growth types range from moist bacterial or yeast-like colonies to the dry, rough growth typical of *M. tuberculosis* var. *hominis*. The growth rate on original isolation varied from 3 days to several weeks and the color from white to deep orange. The morphology of these organisms ranged as widely, from cocco-baccilary forms to the branching filaments characteristic of the genus *Nocardia*.

The objective of our study was to develop a system which was sufficiently simple in medium, technique and time requirements for routine use, and which would yield at least group identification. The problem was approached by a comparative study of identified organisms to establish their cultural patterns on common laboratory media.

Methods. Twelve stock cultures were selected for study, eight *Mycobacterium* species (*tuberculosis* var. *hominis, tuberculosis* var. *bovis, avium, smegmatis, phlei, fortuitum, lactiola,* sp. ATCC # 9031) and four species of *Nocardia (asteroides, madurae, brazilicusis,* sp.). Six of the common laboratory media were found to permit grouping of these stock cultures and to indicate species identification in some. The media are Penassay broth, glycerol agar slants (nutrient agar with 7% glycerine), Littman oxgall agar, corn meal agar, Eosin Methylene Blue agar and blood agar (tryptose blood agar base with 5% blood).

All cultures were inoculated into Penassay broth and incubated at 37C. The time required for production of sufficient inoculum varied from five days for the species showing surface growth, to ten days for those with bacterial type growth.

Centrifuged sediment of the growth in Penassay was inoculated by sterile swab onto Littman's, EMB, blood and corn meal agar plates and incubated at 37C. Swabs were pressed out before streaking to prevent splatter or spread

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of inocula, but isolated colonies are not essential. For preservation of cultures similar inoculum was placed on glycerol agar slants which supported growth within ten days at 37C of all species except *M. tuberculosis* var. *hominis and N. madurae.*

Results. The growth of the cultures in the Penassay broth divided the organisms into four distinct groups. (1) *M. tb.* var. *hominis* and *N. madurae* failed to show visible growth in the medium in ten days although the recovered organism were viable on transfer. (2) *M. tb.* var. *bovis* and *M. lacticola* developed a bacterial type of suspension and sediment. (3) *M. fortuitum* and *N. braziliensis* formed delicate, easily broken surface growth with light frost on the tubes and large amounts of loose sediment. (4) *N. asteroides, N. sp., M. avium, M. smegmatis, M. phlei* and *M. sp.* formed tough pellicles with opaque frost on the glass and small amounts of loose sediment.

Table I records the growth appearance on the respective solid media. The surface growth on corn meal agar offered little assistance in identification. However, this medium allowed easy examination of the respective growth patterns through the bottom of the dish with low microscopic power. All of the *Nocardia* species presented a characteristic pattern of loose, extensive, random branching. *M. fortuitum* showed a distinctive colony edge of multiple central stems with side arborization. The other organisms *M. smegmatis, M. phlei, M. avium* and *M. sp.* presented a non-distinctive appearance.

TABLE I

PENNASSY BROTH 2—10 DAYS 37° C

		Glycerol	Littman	Cornmeal	EMB	Blood
No visible growth	M. tb. var. hominis H37Rv	—	—	—	—	—
	N. madurae	—	—	—	—	—
Bacterial	M. tb. var. bovis ATCC9834	white	—	—	—	—
	M. lacticola ATCC 9626	yellow	—	—	—	—
Delicate pellicle and frost	M. fortuitum ATCC 6841	white moist	white moist	white moist	opaque moist	white moist
	N. braziliensis (Mexicana)	yellow leather	— —	yellow and chalk	flat dry	depressed leathery
Tough pellicle and frost	N. sp.	yellow leather	— —	yellow and chalk	flat dry	brown grainy
	N. asteroides	yellow leather	— —	yellow and chalk	flat dry	chalky surface
	M. avium ATCC 4676	white dry	—	white dry	film	grey moist
	M. sp. ATCC 9031	tan	white sparse	yellow dry	film	yellow moist
	M. smegmatis R#3	grey dry	white dry, rough	white dry	flat dry	white dry
	M. phlei R 34	yellow dry	yellow dry, rough	yello dry	flat dry	yellow dry

In the charted results (Table I) we see that *M. tb.* var. *hominis* and *N. madurae* failed to grow on any of these media in 10 days. *M. tb.* var. *bovis* and *M. lacticola* produced bacterial growth in Penassay broth and were separated from each other by color on glycerol. *M. fortuitum and N. braziliensis* formed delicate pellicles in broth but were easily distinguished on solid media. Those cultures forming persistent pellicles are also distinguishable by this scheme. The Nocardias failed to grow on Littman's which is a genus characteristic as is the branching visible on corn meal. They were yellow on glycerol and corn meal, dry on blood, and *N. asteroides* was identifiable by its prompt and distinctive chalky growth on blood. *M. avium* and *M. sp.* were similar with the exception of color and the inability of *M. avium* to grow on Littman's agar. *M. smegmatis* and *M. phlei* separated only by their respective colors.

When this scheme was applied to the collection of "atypical" acid-fast organisms isolated in Cook County Hospital we were able to identify 11 *N. asteroides,* 26 *M. fortuitum,* and one *M. smegmatis.* The rest of the cultures, around 250 at last count, fit none of the labelled cultures. They were all slow growing, taking from 7 to 14 days to show growth on glycerol agar. They produced the bacterial type of growth in Penassay broth but were not identifiable with stock cultures of *M. tb.* var. *bovis* or *M. lacticola* which showed this pattern. 38 of them were true chromogens, producing deep yellow or orange pigment always on all media. Fifteen of them were truly nonchromogenic, producing no color except a little browning with extreme age. The rest were of the group called photochromes by Dr. E. H. Runyon of Sun Mount VA Hospital because they grow white until exposed to light. Then even a brief exposure caused them to turn bright yellow and retain that color. While these did produce bacterial growth in broth and grew on glycerol agar in 10-14 days, their slow growth made the described media worthless for their further identification.

At this point we turned to intensive study of two organisms *N. asteroides* and *M. fortuitum.* Fifteen isolates of *N. asteroides* were studied by the above scheme with uniform results. Comparisons validated the usefulness of the pellicle-formation on broth, lack of growth on Littman's, and the characteristic branching on corn meal, as identifying characteristics for the genus *Nocardia.* While not all named species of this genus have been tested as yet, the work to date indicates the immediate appearance of a chalky surface on blood agar to be a specific characteristic of the species asteroides. All isolates tested were pathogenic for mice.

M. fortuitum interested us particularly because of its previously reported pathogenicity for man and its unexpected frequency in patients in our hospital. It was found most easily by checking all tb. cultures not more than one week after inoculation, since *M. fortuitum* commonly produced good growth on tb. media in 3-5 days and was strongly acid-fast at any age. The colonies may be bacterial or as rough as true tb. colonies on initial isolation, but are pasty after transfer, grow luxuriantly on all media at 37° C, and remain white except in extreme age. It is highly resistant to heat, dessication and alkali, as is true *M. tb.* var. *hominis.*

The rapid-growth and non-fastidious character of *M. fortuitum* made it easy to perform sensitivity tests by the standard disc and tube dilution methods. The antibiotics commonly used against bacteria were ineffective with the exception of an inhibition of a few strains sensitive to oxytetracycline. However, when the drugs which have been proven effective against *M. tuberculosis* var. *hominis* were tested, the results were striking. Streptomycin and PAS were completely ineffective at therapeutic levels, but INH inhibited all strains at the 1 mcg. level and tetracycline inhibited all strains at the 5 mcg. level. (Table II.)

TABLE II
IN VITRO SENSITIVITY OF M. FORTUITUM

Drug	Conc. (mcg)	Strains Tested	Inhibition	Drug	Conc. (mcg)	Strains Tested	Inhibition
Gantrisin	50	19	0	Oxytetracycline	60	19	8
Penicillin G	10	19	0		10	19	8
Bacitracin	20	19	0	Streptomycin	100	27	0
Neomycin	30	19	0	PAS	25	27	0
Polymyxin	30	19	0	INH*	0.5	27	0
Erythromycin	10	19	0		1.0	27	27
Chloramphenicol	50	19	0	Tetracycline*	1.0	27	0
					5.0	27	27

* Tube dilution method.

Pathogenicity studies further characterized *M. fortuitum.* In mice inoculated intra-peritoneally the liver and spleen are usually abcessed and the lymphatic system widely involved. This same pattern occurs in mice inoculated with *M. tb.* var. *hominis* and with *N. asteroides.* In *N. asteroides,* of course, an acid-fast or a gram stain demonstrates the branching filaments of that genus. However, direct smears of *M. fortuitum* in the pus from a mouse abscess demonstrates acid-fast organisms which are not distinguishable from *M. tb* var. *hominis* in tissue.

In contrast to the pattern of human tb. and *N. asteroides, M. fortuitum* does not infect guinea pigs. Four strains were tested in 6 guinea pigs each, by the system used in our laboratory for pathogenicity studies of *M. tb.* var. *hominis,* and posted at 8 weeks. The only gross lesions found were small abscesses at the site of inoculation in 5 of the 24 guinea pigs. These five abscesses contained AF organisms in the pus, but no organisms were found in other organs. Rabbits were inoculated by the intravenous route without producing any evidence of infection, however, at 60 days after inoculation all rabbits were sensitive to bovine and avian tuberculin and the *M. fortuitum* material. There was no sensitization to O. T. in the 15 rabbits tested.

Summary. In summary, the described cultural scheme is useful in the specific identification of *N. asteroides* and *M. fortuitum,* and in the grouping of other acid-fast bacilli. Its application to a large number of cultures has revealed a significant number of strains of *M. fortuitum.* However, the majority of isolates belong to the slow growing non-chromogenic atypical acid-fast group which remains to be further identified.

VIRULENCE TESTS FOR MYCOBACTERIA*

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Virulence, in the usual accepted sense, implies the ability on the part of the parasite to cause a specific disease in the host either naturally or experimentally. This term is often used interchangeably with the word "pathogenicity"; but in a strict sense, pathogenicity has the meaning of the ability to cause the disease while virulence denotes the measure of the degree of pathogenicity. Regardless of the term used, it should be remembered that disease is not a simple manifestation of the singular property of pathogenicity possessed by the parasite but of the complex of interaction between the host and the parasite.[1] Indeed, virulence as reflected in a disease depends on such factors as the degree of specific and nonspecific host resistance, the physiological states of the host and of the parasite, and various external circumstances attending and influencing pathogenesis. Furthermore, there is a lack of full accord on what constitutes the disease response in the animals injected with different strains of mycobacteria, as a case in point. While this is obviously not a difficulty in those animals that show marked pathologic responses typical of tuberculosis, nor in those with no response whatsoever, responses to those strains which cause from slight to moderate pathologic changes in the host may pose a difficulty in interpretation of their significance, depending on the individual's concept of the significance of such responses.[2] Thus, virulence, although a property ascribed to the parasite, is a term defined in relation to the host and the circumstances affecting it as well as the particular concept of what constitutes a disease response and, therefore, is not an absolute entity.

Classically, virulence has been determined by the use of susceptible animals, i.e. in vivo, and this method has been in use in the study of the tubercle bacillus since its discovery by Koch in 1882. Because of the fairly uniform and consistent response to tuberculous infection and the ease in their handling and maintenance, the guinea pig has long been the animal of choice, particularly adapted to diagnostic work involving the inoculation of suspected clinical material. Although other small animal species, especially the mouse, have been used advantageously in such specialized procedures as screening various drugs for their antimycobacterial activity,[3] assaying the effectiveness of antituberculosis drugs,[4] and determination of certain immune responses,[5,6] they have not proved to be satisfactory for routine use.[7] Virulence in the guinea pig, however, of different strains of tubercle bacilli has not always been consistent and correlation between the severity of human disease and animal response has been far from satisfactory.[1] This difficulty is especially apparent with the atypical acid-fast strains since many of them show little or no effect in the guinea pig.[8,9,10] On the other hand, it has been shown that the mouse is considerably more susceptible to injection of some chromogenic acid-fast strains than is the guinea pig.[9b,10,11] Mycobacterium *ulcerans*, for example, has been demonstrated to be quite infectious for the rat and mouse while the guinea pig is insusceptible.[12,13] Different mice

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strains, however, are considerably different in their susceptibility to inoculation of acid-fast bacilli;[14,15] age of this animal is another factor which influences susceptibility.[15] The Syrian golden hamster has been used to a lesser extent in testing the virulence of certain chromogenic acid-fast strains,[9b] but as yet widespread use of this animal for routine work has not been noted, although its use has been recommended.[9b,11]

In inoculating the guinea pig or other animals for virulence testing, certain conventions which have come to be accepted as standard conditions by virtue of their long use are followed.[16] The route of inoculation for the guinea pig is usually subcutaneous in the inguinal area, and in an infected animal not only are the local or regional lymph nodes involved but subsequent progressive infection is seen in other lymphatitc tissues, partitcularly in the spleen, liver, and lungs as well as in the distant lymph nodes in the omentum, mesentery, the retroperitoneal region, and the tracheobronchial sites. Thus, the outstanding characteristics of tuberculous infection in the guinea pig is the extensive involvement of the lymphatic tissues which are apparently reached by lymphatic dissemination. Unlike the guinea pig, the mouse is usually injected intravenously in one of the lateral caudal veins for the most uniform response, although the intracerebral route is said to result in greater consistency. Because of the intravenous route of inoculation, the lesions are seen grossly in the lungs and frequently in the liver, but not in the spleen and kidneys, although acid-fast bacilli may be found during the early and terminal stages of the disease.[17,18] Splenic hyperplasia is usually present. One strain of acid-fast bacilli, *Mycobacterium fortuitum* Cruz,[19] is said to give rise to kidney lesions only and this has been suggested by Wells et al. as a specific diagnostic characteristic of this species.[20]

In addition to the route of inoculation, the amount of the inoculum has considerable influence on the outcome. This is noticeable especially when the intravenous route is used. Indeed, under carefully controlled conditions, the median survival time of mice so inoculated when plotted against the logarithm of the amount of the inoculum shows a relationship that is approximately linear.[21] Moreover, the inoculum itself, i.e. the acid-fast organisms, may show variations in virulence due to differences in the medium on which it was grown. Such variation in virulence of a standard human strain, H37, has been noted and documented.[22] However, this problem has become a relatively minor one in this day of quality control of various ingredients that are used in pre-packaged media formulations and the use of semi-synthetic media such as the Dubos Medium.

Because of the advantages of simplicity, ease, economy, speed, and cleanliness of the procedure offered by the in vitro methods, they have long attracted the attention and interest of those who have been engaged in the study of the tubercle bacillus. Early attempts to correlate both the microscopic and cultural morphology with virulence have been abandoned because of inconsistent results.[1] A more sophisticated approach has been the biochemical one but the earlier ones have not withstood the test of reliability and have been forgotten. It would appear that to date the difficulty with most of the in vitro tests for virulence lies in their basis since most of them are designed not to determine virulence per se but only the measurable properties or characteristics which are found in, or associated with, virulent acid-fast bacilli. Therefore, the problem is one of statistical correlation between virulence and the measured char-

acteristic which in itself in most instances appears to have no role in pathogenesis.

Among the earlier tests devised along these lines is the one based on the ability of the avirulent strains to reduce methylene blue to its leuco or colorless compound. This methylene blue reducing capacity was noted to occur at a slower rate[23] or was absent[24] in the virulent strains. An extension of this test is the measurement of the oxidation-reduction potential differences between the avirulent and virulent strains, using different indophenol or chlorphenol dyes as indicators that show color changes at different oxidation-reduction potential levels.[25] Both of these tests and the modification of the latter one[26] have been consistent for most virulent strains, but few of the known avirulent acid-fast bacilli have given erratic and inconsistent results. Because of these false results these in vitro tests were deemed to be unreliable as substitutes for the in vivo test.[27,28]

In 1948 Dubos and Middlebrook described a cytochemical test using the neutral red dye.[29] In this test the virulent bacilli bind the neutral red dye in an alkaline buffer solution, turning the methanol-washed cells to a pink or red color. In contrast to the virulent strains, cells from many avirulent strains were found to be incapable of binding the dye under the conditions of the test. Whether or not the neutral red dye binding capacity is somehow related to pathogenicity has not been established, but the correlation between the virulent strains and the neutral red dye binding capacity is well established.[30,31] However, there is room for doubt as to whether or not the avirulent strains are all incapable of binding this dye. Indeed, the accumulated evidence is less satisfactory than for the virulent strains and has led Richmond and Cummings to conlude that the guinea pig inoculation was a more reliable test for virulence than the cytochemical test.[32]

Another characteristic associated with virulence but occurring under special circumstances is the catalase activity present in most virulent strains and lacking in isoniazid-resistant, avirulent strains.[33] However, not all avirulent strains are devoid of the enzyme catalase and not all isoniazid-resistant mycobacteria are catalase negative. The most that can be said at present is that many isoniazid-resistant, catalase negative strains of acid-fast bacilli are avirulent for guinea pigs.[34,35] As yet the significance of catalase activity is little understood.

Perhaps the most interesting and in some respects the most promising characteristic of the virulent strains is their morphology of the microcolonies. Middlebrook, Dubos, and Pierce described the growth pattern of the virulent strains as forming a cord and called it the "serpentine cord" formation.[36] The correlation between cord formation and virulence in the guinea pig has been confirmed by others[37] and Bloch isolated a specific chemical entity now known as the cord factor.[38] Its chemical structure has been partially analyzed and it is known to be composed of an alpha-mycolic acid moiety and carbohydrate fraction containing an atom of nitrogen.[39] Bloch attributes most of the previously described characteristics associated with virulence to the presence of the cord factor, although the cord factor itself has only a limited biological activity in mice and probably none in the guinea pig.[39]

More recently Krasnow et al. devised a test designed to incorporate neutral red dye binding and cord formation tests in a single in vitro test

for virulence.[40] On a theoretical basis the probability of two characteristics, assuming their independence to each other, associated with virulent strains occurring in an avirulent strain is considerably less than the occurrence of one of these characteristics. The test is based on the ability of the acid-fast strains to grow microcolonies on filter paper.[41] Goldman and Goldman, however, reported that depending on certain growth conditions an avirulent variant, Strain H37Ra, was capable of growing in cords as well as binding the neutral red dye.[42] Furthermore, they discovered previously that a contaminating bacterial colony identified as *Micrococcus epidermidis* produced a soluble factor in the medium inducing the formation of cords in the avirulent variant strain of the acid-fast organisms growing in the vicinity of the micrococcal colony.[43]

Hence, the present status of the cord factor appears to be that it is a chemical entity occurring in most virulent strains. It may directly influence other characteristics usually found in, or associated with, the virulent strains, as suggested by Bloch,[38] but in itself it is not the virulence factor.

When the various findings are considered, it would appear that the in vivo test must still provide the final answer to the question of virulence. The in vitro tests discussed here are suggestive and helpful but not conclusive of, or exclusive for, virulence; at best they may be used to yield corroborative evidences of virulence. It is hoped that factor(s) responsible for virulence and measurable in vitro may be found, but as yet that discovery remains for the future.

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AN IMMUNOLOGICAL METHOD FOR QUANTITATING SERUM GAMMA GLOBULIN*

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In 1952 after Bruton[1] first described agammaglobulinemia as a new disease entity, it became increasingly desirable to find a sensitive method of quantitating low concentrations of serum gamma globulin.

At that time, one could obtain serum gamma globulin concentrations by the following methods: (1) salting-out techniques, such as those of Jager and Nickerson,[2] and Wolfson, Cohn and associates;[3] (2) zinc turbidity measurements as devised by Kunkel;[4] (3) free electrophoresis; (4) paper electrophoresis.

Both salting-out techniques depended upon the precipitation of protein from ammonium sulfate solutions and subsequent color development with biuret reagent. Precipitates prepared by each of these methods were found to be electrophoretically impure, the Jager and Nickerson precipitates ranging from 73-83% gamma globulin and those of Wolfson, Cohn et al. showing 90% gamma globulin. Results by the method of Wolfson and associates were shown to correlate fairly well with values obtained by free electrophoresis,[5] although no data have been reported for hypogammaglobulinemic sera and behavior under these conditions is not well known. Both of the salting-out methods had been used particularly for sera having normal or increased amounts of gamma globulin.

The zinc turbidity test of Kunkel was not devised for measuring low concentrations of gamma globulin and is not sufficiently quantitative for use at these levels.

Free electrophoresis has been considered the standard of reference for quantitating values of gamma globulin in sera having normal or increased concentrations. Paper electrophoresis is now established as a more convenient technique; values for gamma globulin concentrations compare favorably with those obtained by free electrophoresis.[6] However, neither electrophoretic method is satisfactorily accurate for measuring the reduced values for gamma globulin from hypo- and agammaglobulinemic sera; we have observed errors in measurement as great as 100-200%.

Therefore investigators have turned to immunological techniques to obtain a more accurate measure of low serum gamma globulin concentrations. Wiener has described an erythrocyte agglutination-inhibition test[7] and later applied this to sera of agammaglobulinemic patients.[8] By a similar method, Grubb[9] has detected as little as 0.1 micrograms of gamma globulin. Though excellent for identifying small amounts of gamma globulin, accurate precision in quantitation has not yet been claimed for these techniques.

The precipitin method to be described herein depends upon the separation of gamma globulin from serum samples with specific antisera, and quantitation of the antigen-antibody complex by means of a modified Folin method.

* Presented in part before the Silver Anniversary Convention of ASMT, Chicago, Illinois, June 1957.

Method

Preparation of the antigen:

The antigen used for preparing the antiserum was Cohn Fraction II prepared from fresh human plasma in our laboratory, using Cohn Methods 6 and 9.[10] This preparation gave an electrophoretically homogeneous and symmetrical peak. Commercial gamma globulin prepared in the same fashion may be similarly used.

Preparation of the antiserum:

Ideally, male rabbits weighing at least 4 kilograms are injected in the marginal ear veins with alum-precipitated gamma globulin, prepared according to Kabat and Mayer.[11] A series of injections are given 3 to 4 times weekly, administering a total of from 7.5 to 10 mgms. of gamma globulin per kilogram of body weight, over a period of 30 days. A few days after the last injection, rabbits showing adequate antibody titre are exsanguinated. The sera are pooled and heated at 56° C. for 30 minutes to destroy complement. After checking for the presence of contaminating antibodies, and removing these by absorption if necessary, merthiolate is added to a final concentration of 0.01%. The sera are bottled in small vials and stored in the frozen state.

The antiserum employed for our study presented herein was obtained from rabbits which had had three courses of gamma globulin injections and consequently had small amounts of contaminating antibodies. These were removed by absorption with serial additions of an agammaglobulinemic serum,* centrifuging to remove precipitate after each addition. Tests of aliquots of the pool indicated adequate antibody absorption when 0.8 ml. of agammaglobulinemic serum was added to 570 ml. of pooled rabbit serum.

Tests of antiserum purity:

Since a pure antiserum is an essential for accurate determination with an immunochemical method, final tests for antibodies to serum components other than gamma globulin were carried out by various agar techniques, including Oudin tube, Ouchterlony plate and immunoelectrophoresis.

Ouchterlony agar plates[12] were set up using anti-gamma globulin serum in one well, pure gamma globulin in the second well and whole human serum in the third (Figure 1). After diffusion of these components into the agar, lines of specific precipitate formed between the known gamma globulin and the anti-gamma globulin serum, and also between the anti-gamma globulin serum and the whole serum. Since these two lines met but did not intersect, both lines resulted from a common antigen-antibody system. The absence of additional lines between the anti-gamma globulin serum and the whole human serum strongly suggested there to be but one antigen-antibody system, though it was yet possible that other antibodies existed in extremely low titre.

Following the immunoelectrophoretic method of Grabar and Williams,[13] whole human serum was placed in a well in a plate of agar and the electrophoretic components were distributed by direct current throughout the gel (Figure 2). After electrophoresis, troughs were cut in the gel plate on each side of and parallel to the axis of electrical migration of the serum proteins. In one trough was placed the anti-

* Kindly furnished by Dr Robert A. Good, University of Minnesota

gamma globulin serum to be tested, mixed with agar, and, in the other, anti-whole human serum mixed with agar. The lines of specific antigen-antibody precipitate appeared in 4 to 6 days, showing one precipitin system on the side of the anti-gamma globulin serum and as many as ten different precipitin systems on the side of the anti-whole human serum. It was regularly noted that the line of gamma globulin precipitin extended the length of most of the plate, including the regions of alpha and beta globulins, in accord with the previous observations of Grabar and Williams.[13] The lines of the other serum components were of far more limited range.

Calibration curve and mechanics of quantitation procedure:

The calibration curve was prepared using a solution of the same pool of lyophylized gamma globulin that had been used for the initial immunization. Protein content was determined by micro-Kjeldahl nitrogen analysis.[14] This stock solution was then diluted with saline to give a final concentration of 12 mgms.%.

Figure 1

Ouchterlony plate, demonstrating homogeneous antigen-antibody system.

0 = Anti-gamma globulin serum
1 = Gamma globulin
2 = Whole human serum

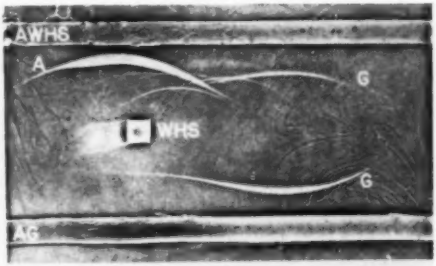


Figure 2

Immunoelectrophoretic agar plate, comparing the homogeneous antigen-antibody system from gamma globulin antiserum (below) with heterogeneous system from antiserum made to whole human serum (above).

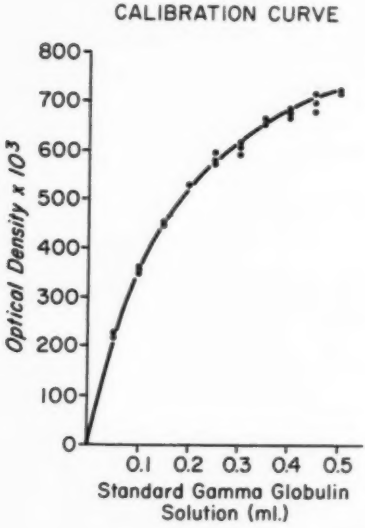
WHS = Whole human serum
AWHS = Anti-whole human serum
AG = Anti-gamma globulin serum
G = Gamma globulin precipitin line
A = Albumin precipitin line

A series of 12 ml. heavy duty centrifuge tubes were set up in triplicate. To each of these was added a 0.4 ml. aliquot of antiserum and a measured quantity of the standard gamma globulin solution. These solutions were mixed immediately and thoroughly after addition of gamma globulin. The total volume in each tube was adjusted to 4 ml. with saline. The tubes were allowed to stand one hour at room temperature and were then stored 48 hours at 4° C. The tubes were centrifuged one hour at 3200 rpm (in an International refrigerated centrifuge, model PR-1, head model 831) at 4° C., the supernatant fluid decanted, and tubes inverted and drained for one hour

in the cold. After flicking the tubes to break up the precipitate, the sediments were washed with small amounts of cold saline. Centrifuging was then carried out at 3200 rpm for 15 minutes at 4° C. and the tubes decanted and drained for 30 minutes in the cold. This washing cycle was repeated.

The precipitates were dried over P_2O_5 in vacuo for half an hour. Nitrogen contents were determined colorimetrically by the method of Folin-Ciocalteu as modified by Lowry and associates.[15] The precipitate of a single tube was dissolved in 0.2 ml. of 1 N NaOH. After standing for 30 minutes, 2 ml. of Reagent D were added, followed ten minutes later by 0.2 ml. of Folin-Ciocalteu reagent, with immediate and thorough mixing. Colors were allowed to develop for 30 minutes, 2 ml. of distilled water were added and the colors read at a wavelength of 500 $m\mu$ in a Beckman Model B spectrophotometer. Matched square Leitz cuvettes of 1 cm. light path, cut down to fit the Beckman 4-unit carrier, were employed. The zero reading was adjusted to a blank of antiserum which had been carried through the whole procedure.

The calibration curve was constructed using optical density x 10^3 as ordinate and ml. of standard gamma globulin solution as abscissa. In making the calibration curve, the color was found to bear a linear relationship to protein concentration only up to 0.30 O. D. (Figure 3). For this reason, the curve was expanded in this area (Figure 4). All samples should be diluted to allow readings within the range of linearity.



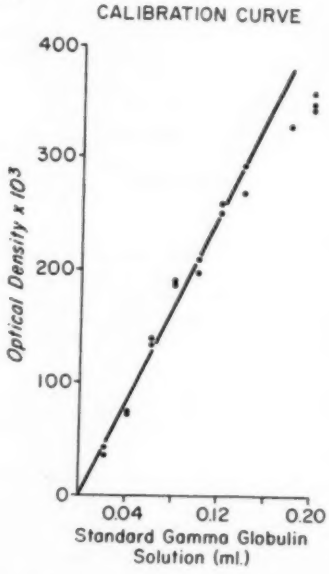


Figure 3
Reference curve demonstrating non-linearity above 0.3 O. D.

Figure 4
Reference curve, showing linearity below 0.3 O. D. Standard gamma globulin solution is half the concentration of that shown in Figure 3.

Sera to be tested were handled in the same manner and results were calculated from the calibration curve according to the following formula:

$$\text{Concentration of gamma globulin in sample in mgms. \%} = \frac{d(C \times \text{ml. standard gamma})}{\text{ml. of diluted serum}}$$

in which C = concentration in mgms. % of the standard gamma globulin solution

d = dilution factor of the unknown serum

Routinely, normal sera were diluted 1:100 and 2 sets of triplicates were run, using aliquots of 0.05 and 0.1 ml. of diluted serum, averaging the results. For low sera, the amount of dilution necessary was estimated from a paper electrophoretic strip, and two different aliquots were always used as before.

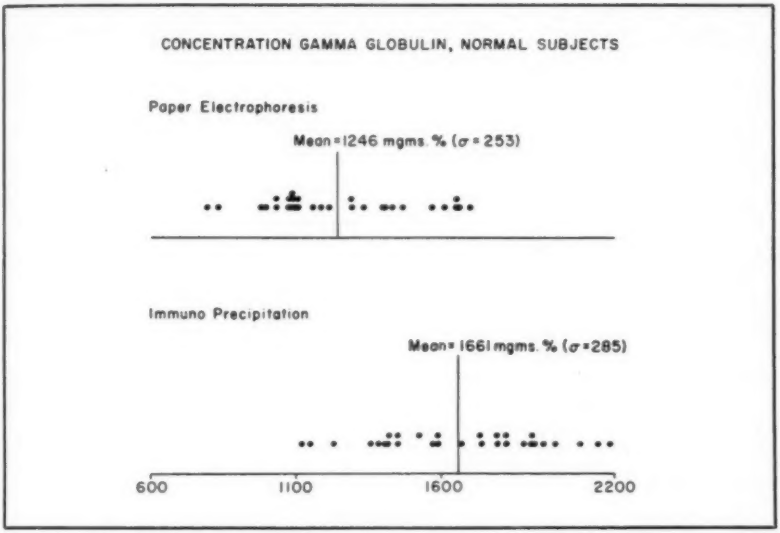


Figure 5

Results

Quantitations were obtained on two groups of sera: (1) patients who had varying degrees of hypogammaglobulinemia; (2) normal adults, ranging in age from 22 to 78 years.

The ten sera in the first group ranged from 3 to 800 mgms. % gamma globulin.

Normal values, based on the analysis of 30 sera, were determined by both the immunoprecipitin method and by paper electrophoresis*, with subsequent scanning of the paper strips with a Spinco Analytrol. Normals for the immunochemical method with our antiserum** were found to range from 1100 to 2200 mgms. % with a mean of 1661, while values on these same

* The paper electrophoretic analyses were performed according to the Spinco procedure,[34] with the exception that the ionic strength of the veronal buffer used was 0.10 μ. This necessitated a running time of 19 hours with a current output of 8 MA for 1 cell.

**Increased valence to certain of the antibodies may falsely elevate precipitin values obtained with antisera resulting from repeated courses of antigen injections.

sera by paper electrophoresis ranged from 790 to 1700 mgms. % with a mean of 1246 (Figure 5).

In a series of 251 sets of triplicate determinations, the coefficients of variation were 5 or less in 90% of the determinations and 2 or less in 53%.

Coefficients of variation for determinations carried out on aliquots of the same 4 hypogammaglobulinemic sera on successive days were as shown in Table 1. Each of these coefficients is calculated from the mean value of five daily determinations. Each figure in the first column is an average of six determinations.

TABLE 1

COMPARISON OF DAILY DETERMINATIONS, HYPOGAMMAGLOBULINEMIC SERA

Patient	Mgms. %	Mean	Standard Deviation	Coefficient of Variation
S—H.	168 150 165 170 150	161	8.8	5.51
R.	189 192 198 213 217	202	11.2	5.57
P.	411 425 440 425 418	424	9.6	2.27
H.	514 498 499 556 593	532	37.8	7.12

Discussion

The gamma globulins represent a heterogeneous group of plasma proteins. Electrophoretically defined, they consist of those components migrating with mobilities of -0.97×10^{-5} to -2.63×10^{5} cm²/volt/sec when the buffer used is composed of diethylbarbituric acid and sodium barbital, pH 8.6, ionic strength 0.1.[16] A normal range of 600 to 1600 mgms. % has been found by free electrophoresis.[17, 18] Good correlation of values for gamma globulin determined by free and paper electrophoresis in normal subjects has been shown.[6]

Immunologically defined, the gamma globulins consist of those plasma proteins which react with antiserum prepared from an electrophoretically homogeneous gamma globulin. Values for normal subjects obtained by immunologic reactions may be considerably higher than those determined electrophoretically, for two reasons: (1) electrophoretically, some gamma globulins may be pulled into alpha and beta globulin positions through protein interaction with components of faster mobility,[19] and thus are omitted in the analysis of the gamma globulin peak of schlieren diagrams; (2) certain plasma proteins normally migrating in the alpha and beta globulin positions

react immunologically as gamma globulins.[20] As studied by immunoelectrophoresis, these proteins appear to participate in the single homogeneous antigen-antibody reaction for gamma globulins[20, 21] (Figure 2).

Thus, immunochemically determined values for gamma globulin concentration of all sera, even though obtained with pure antisera, should be greater than the corresponding values obtained by electrophoresis if the antigen employed for producing the antiserum contains a broad distribution of the gamma globulins. With the precipitin method herein reported, our values for gamma globulin concentration of normal sera are nearly a third higher than their corresponding paper electrophoretic range.

We are aware of the normal range for serum gamma globulin of 1020 to 1380 mgms. % reported by Goodman and associates,[22] employing a similar immunoprecipitin method and chicken antiserum. Good and associates[23] have recently reported normal values of 700 to 1300 mgms. % using rabbit antiserum. However, with the use of immunochemical methods, results may vary from laboratory to laboratory, depending upon the antigen employed, the schedule for immunization, and the animal species in which the antiserum is developed. Indeed, in the same laboratory each new pool of antiserum must be calibrated and a series of normals run to determine its normal range.

For hypogammaglobulinemic sera, this method shows excellent sensitivity, and the reproducibility is considered superior to those experiences described for the erythrocyte agglutination-inhibition test by Wiener[7, 8] and by Grubb.[9] The sensitivity of the precipitin test in differentiating various degrees of hypogammaglobulinemia is vastly superior to free or paper electrophoresis.

Summary

An immunologic precipitin method has been described which sensitively measures low concentrations of gamma globulin in human sera.

For the successful use of this method, one must have: (1) a pure gamma globulin as antigen, preferably one containing most of the components of the gamma globulin complex, and (2) a pure antiserum. Great care should be taken in ascertaining antiserum purity and in absorbing out contaminants.

Normal values differ from those found by other types of methods for determining gamma globulin, but the actual proteins measured also differ somewhat in their identity.

Acknowledgements

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LISTERIA MONOCYTOGENES—A STRANGER IN YOUR LABORATORY?*

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Early in 1954 we made a survey of the reported cases of listeriosis. We found a total of 40, indicating that the disease is rare. A search of the U. S. literature just prior to this paper yielded two cases reported from Minnesota,[1] one from Virginia,[2] one from Missouri,[3] two from Maryland,[4] one from Washington, D. C.,[5] one from California,[6] one from New York,[7] and ten from Louisiana.[8] The Communicable Disease Center reports a total of 52 positive cultures received from 1950 through August, 1956,[9] and two cases are reported in Morbidity and Mortality, U. S. Dept. of Health, Education and Welfare.[10] It is the contention of the authors and others that the disease occurs much more frequently than these figures indicate, but that the causative organism, *Listeria monocytogenes*, is a stranger in most laboratories. Because it closely resembles our friend the diphtheroid, it is discarded without being recognized as a pathogen. Although some of those reporting cases since 1954 still attribute the paucity of the cases to the rareness of the disease, we feel that the responsibility for this rareness rests with the laboratory. Reed states, "It is all too easy in a busy laboratory to label a gram positive rod in cerebrospinal fluid as a contaminating diphtheroid."[11] Murray, the first to isolate the organism, recently wrote, "The actual diagnosis depends upon the alertness of the bacteriologist."[12]

The possible lack of alertness is quite understandable. The usual laboratory references do not stress the importance of the organism, and many of them completely ignore it. Practicing medical technologists must be made aware of the existence of the organism and alerted to the possibility of its presence. This paper is presented to you with the hope that general dissemination of information will lead to the diagnosis of many new cases.

May we explain that we have not always known this organism. Our first contact was a patient admitted from an out-of-town hospital. A specimen of cerebrospinal fluid accompanied the patient. Our culture of this fluid showed an organism we could not identify, and since we thought it questionably gram negative, we hesitated to call it a diphtheroid. We sent it to our local branch laboratory of the Michigan Department of Health for identification. Records showed that the state laboratory had also received a portion of the original spinal fluid, and that the culture had been discarded as a diphtheroid and probable contaminant. This is not to be construed as criticism of the work of the state laboratory, but is given to you, with their permission, to point out that the organism may not be recognized by a laboratory doing much more bacteriology than the average hospital laboratory. It was at the state laboratory that, after our culture was received, the suggestion was made that the organism might be *Listeria monocytogenes*. Our answer to that suggestion was a very inelegant "what's that?," which has also been

* 3rd Bacteriology SPF Award. Presented before 25th Anniversary ASMT Convention, June 1957, Chicago, Illinois.

the general reaction when we have attempted to introduce the organism to others. Our efforts to learn something of the organism from the textbooks yielded but little. The longest description was found in Zinsser's Textbook of Bacteriology[13]—a half page. We are happy to find that this half page has been expanded to a full page in the tenth edition.

The organism was first isolated by Murray in England and Pirie in South Africa in 1924. It has been called *Bacterium monocytogenes, Listerella hepatolytica,* and *Listerella monocytogenes.* The original isolations were made from rabbits and other domestic and wild animals. In rabbits, guinea pigs and mice, it produces an increase in the mononuclear leucocytes, the reason for the monocytogenes portion of the name. In cattle and rabbits *L. monocytogenes* may produce abortion, while in sheep, goats and other animals it may develop into a septicemia with necrotic foci in many organs, and cerebral involvement resulting in what is known as "circling disease." The most common infection in humans is a meningo-encephalitis. It may occur at any age, but is most frequently seen in newborns and infants. The clinical symptoms are similar to other types of meningitis or encephalitis. The laboratory findings in the cerebrospinal fluid include an increase in the leucocytes, largely polymorphonuclears, an increase in protein and decrease in sugar, and usually a positive culture. In some cases, a positive blood culture may be found when the spinal fluid culture is negative.

Bergey's Manual lists this organism as the cause of infectious mononucleosis. This has not been confirmed to the satisfaction of most workers. One of the reasons for non-acceptance has been because of the inability of the organism to stimulate the formation of sheep erythrocyte agglutinins in the rabbit. The first human isolation of the organism was made in 1929 by Nyfeldt[15] from a boy suffering from a syndrome resembling infectious mononucleosis. Other workers have reported the isolation of *L. monocytogenes* from cases of infectious mononucleosis but the findings are not consistent. Stanley, an Australian author quoted by Girard and Murray[16] advances the hypothesis that infectious mononucleosis can be classified into three groups on a serological basis. The infections falling into Group A may be due to *L. monocytogenes.* This group A comprised 35% of the 20 cases he studied.

Listeria monocytogenes is a small gram positive rod with rounded ends, having a tendency to show coccal forms or shorter rods. It decolorizes easily and tends to become gram negative in older cultures. It does not produce spores. It grows well on human blood agar, tryptose phosphate broth, thioglycollate broth and chocolate agar. The colonies are small, smooth and gray, and show beta hemolysis with indistinct edges. The organism is motile, more so at room temperature than at 37°, and shows a rolling, tumbling motion. The reactions in carbohydrates are variable. The organisms we have isolated fermented dextrose and maltose, but did not ferment sucrose, lactose, sorbitol, xylose, mannite and dulcite. No gas was produced in any carbohydrate. Hydrogen sulfide and indol were not produced, and the Voges Proskauer reaction was variable.

While absolute confirmation of diagnosis is dependent upon serological studies, one rather simple laboratory procedure is available to most technologists. A saline suspension of the organism is dropped into the

eye of a rabbit, using the technique suggested by Julianelle.[17] Conjunctivitis quickly develops. If a rabbit is injected intravenously with the same saline suspension, it usually dies within 48 hours. Pneumonic lesions can be seen in the lungs and the organism recovered from these lesions. The typical production of monocytes may be observed in smears of the peripheral blood.

Much of the current literature on *L. monocytogenes* comes from Germany where the disease is endemic. Many of the cases have been in newborns. This leads to the inevitable question as to whether there is a transplacental infection either from contact by the mother with an animal source or from the use of unpasteurized goat's or cow's milk. Potel[18] working in Germany isolated *L. monocytogenes* from the milk of a cow with atypical mastitis. A woman drinking milk from the cow delivered twins prematurely and *L. monocytogenes* was isolated from the liver of each. Murray states that "listeriosis seems less like a disease transmissible from animals to man than like an infection which man shows with other animals an equal host susceptibility."[12] Certainly much more work needs to be done to establish the true role of *L. monocytogenes* in infant deaths. At the suggestion of Dr. William Cherry of the Communicable Disease Center we have been taking cultures from the stomach, upper and lower intestines of all stillborns and neonatal deaths in an attempt to isolate the organism. So far our results have been negative. Our maternity cases come primarily from an urban population. Too, there are rigid state regulations regarding pasteurization of milk, even in rural areas. We would like to suggest that others, particularly those having access to cases in areas where raw milk is used, attempt the same sort of study. The study is facilitated by the fact that refrigeration does not kill the organism, in fact growth may be enhanced. Gray, et al,[19] have shown that spinal fluid and macerated organs of animals, if kept at 4° C for from two to three months, will yield positive cultures when ordinary methods at room temperature or 37° C were negative.

In April of this year we received a routine culture from a neck abscess. We were greatly surprised to have the organism cultured from it prove to be *L. monocytogenes.* The cultural characteristics were typical. Conjunctivitis was produced in the eye of a rabbit, and a marked monocytosis was demonstrated in the rabbit injected intraperitoneally. The organism was recovered from the lungs of the latter rabbit. Our findings were confirmed by the Communicable Disease Center.

It was learned that the patient had been treated by an osteopathic physician for a month prior to his admission to the hospital. He had received several injections of a procaine derivative for a wrenched right shoulder suffered at work in a dry goods warehouse. The abscess was localized below the right ear. In a list of 287 cases of world wide distribution compiled by Seeliger[20] in 1955 we were unable to find a case in which the infection was so localized as in this patient.

To summarize the cultural characteristics:

1. It grows well on ordinary liquid or solid media, particularly blood agar, on which it shows definite but indistinct hemolysis.
2. Carbohydrate fermentations with the exception of dextrose are variable.

3. Motility studies at room temperature and at 37° C show greater action at the lower temperature, with a peculiar rolling or tumbling motion.
4. The production of conjunctivitis in the rabbit is, perhaps, the easiest and most reliable diagnostic test.
5. Serological identification is the final step in confirmation. The CDC will accept cultures for identification, or perhaps you will find a college of veterinary medicine in your area is interested in the problem and able to assist you.

In conclusion we feel from the study of our cultures and from our search for more information about the organism and the disease it causes, that infections with this organism are more common than reports would indicate. Furthermore, since meningo-encephalitis seemingly is the most common form in this country, we believe that any gram positive or gram variable organism resembling a diphtheroid which has been isolated from cerebrospinal fluid should be considered as possible *Listeria monocytogenes* until proven otherwise.

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EFFECT OF VARYING AMOUNTS OF POLYSAL AND/OR DEXTROSE* INTRAVENOUS FLUIDS ON THE BLOOD LEVELS OF SODIUM, POTASSIUM, CHLORIDE AND BLOOD SUGAR

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Preamble

The extensive use of various intravenous fluids that are now ordered for the hospitalized patient has become routine. Their value as therapeutic agents is well proven and a great deal of study and research has been focused upon the development of various solutions calculated to treat or support specific physiologic conditions.

This investigation was undertaken to explore the effect of polysal and/or dextrose upon certain laboratory tests if the blood were drawn while the patient was receiving these solutions intravenously. This clinical laboratory, as well as others, noted that occasions were continually arising in which there was a question as to the clinical interpretation of the laboratory's findings. Blood drawn during the time when the patient was receiving an intravenous solution of polysal and/or dextrose or other solutions quite often gave rise to questions by the medical and technical staff as to reliability of the laboratory's findings. The medical technologist, having carefully made the determination as per routine procedure, felt that the discrepancy was due to the fluid being administered intravenously, rather than an inaccurate laboratory procedure.

It was decided that as no other definite study could be found in the available literature, an experiment which would serve as a basis for some deductions relative to the problem was needed. No correlation as to the effect of electrolytes in sugar metabolism, or vice versa, was anticipated.

Experimental Methods and Procedures

The effects of several intravenous solutions on the serum level of sodium, potassium, chloride and the blood sugar level were determined. Specifically, the solutions used are those commonly administered in many hospitals and were as follows:

1. Polysal, a solution containing 140 meq./1. Na, 10 meq./1. K, 5 meq./1. Ca, 3 meq./1 Mg, 103 meq./1. Cl, and 55 meq./1. HCO_3
2. 5% dextrose in polysal.
3. 5% dextrose in water.
4. 5% dextrose in normal saline.

Patients

The patients were in a general hospital of 450 beds and were selected for the study at random. No particular reference as to age, sex, or type of illness was stipulated. The patients were all fasting, before and while the solutions were given, so that the effects of food were negativated. Except for the solution, the amount of solution, and the time element involved in giving the solution, the survey was purposely made as nonspecific as possible.

*Second SPF Award in Chemistry, 1957. Read before 25th ASMT Convention, Chicago, Illinois, June, 1957.

Solutions

The study was made using four collections of blood specimens. These were made under the following conditions:

A. Collections made
   1. Fasting patient.
   2. After 100 cc of solution had been infused.
   3. After 500 cc of solution had been infused.
   4. After 1000 cc of solution had been infused.

B. The time required for the above total amounts to be infused varied from one hour to as much as five hours and was so recorded.

Patient Management and Collection of Specimens

The procedure for collecting blood specimens began by obtaining a sample of the patient's blood just before the intravenous fluid was started. Usually, the same needle used to obtain the primary blood specimen was used for the intravenous infusion. Further specimens were drawn from the opposite arm after the patient had received 100 cc., 500 cc., and 1000 cc. of the infusion. As soon as possible, the blood specimen was returned to the laboratory and the serum removed from the cellular portion of the blood. For the blood glucose determination, lithium oxalate was used as an anticoagulant and the blood used to prepare a protein-free filtrate according to Folin.

Technical Procedure Used

1. Blood Glucose using an Evelyn colorimeter. (1)
2. Serum chloride
   Method: Schales and Schales. (2)
   Modified: 1 cc of the mercuric nitrate titrating solution equals 100 meq./l chloride ion when titrating 2 cc of a 1:10 protein-free filtrate.
3. Serum sodium and potassium using a Baird Flame Photometer.
4. Standard solutions were used in every determination to further standardize the results of each series of tests to a common denominator.

TABULATED RESULTS
5% DEXTROSE IN POLYSAL

PATIENT	I	II	III	IV	V	VI
Infusion Time	2½ Hrs.	3¼ Hrs.	2 Hrs.	3 Hrs.	4 Hrs.	4¼ Hrs.
Blood Sugar in mg %:						
Fasting	122	126	129	141	81	122
100 cc	160	166	172	200	90	115
500 cc	254	192	x	247	115	103
1000 cc	247	240	292	185	218	141
Sodium in meq/l:						
Fasting	137	138	144	124	136	133
100 cc	134	137	138	126	135	127
500 cc	132	136	x	129	132	128
1000 cc	125	135	137	127	134	130
Potassium in meq/l:						
Fasting	4.0	3.9	3.6	4.7	3.5	4.1
100 cc	4.0	3.9	3.7	*	3.6	4.0
500 cc	3.9	3.6	x	5.1	3.6	4.4
1000 cc	3.7	3.4	3.5	4.7	3.4	4.1
Chloride in meq/l:						
Fasting	107	103	106	99	103	77
100 cc	108	104	104	105	109	81
500 cc	106	100	x	100	109	88
1000 cc	99	99	107	101	107	71

x Specimen not satisfactory.
* Hemolyzed serum.

To be noted:

1. The extreme variability of blood sugar concentration irrelevant to time or amount of solution infused.
2. The slight variability of sodium.
3. The stability of potassium and chloride.

5% DEXTROSE IN WATER

Patient	a	b	c	d	e
Infusion Time	2½ Hrs.	5¼ Hrs.	2¼ Hrs.	4 Hrs.	5 Hrs.
Blood Sugar in mg. %:					
Fasting	185	63	122	117	122
100 cc	178	42	200	128	150
500 cc	208	109	234	254	271
1000 cc	141	185	240	118	252

Patient	f	g	h	i
Infusion Time	4¼ Hrs.	4 Hrs.	3½ Hrs.	5 Hrs.
Blood Sugar in mg. %:				
Fasting	133	129	141	115
100 cc	137	137	185	166
500 cc	200	92	271	160
1000 cc	218	112	218	214

5% DEXTROSE IN SALINE

Patient	1	2	3	4	5	6
Infusion Time	5 Hrs.	4 Hrs.	3½ Hrs.	2½ Hrs.	4 Hrs.	4 Hrs.
Blood Sugar in Mg. %:						
Fasting	98	129	106	112	88	86
100 cc	150	166	145	118	98	108
500 cc	133	172	155	126	204	165
1000 cc	200	155	192	133	200	137
Sodium in meq/l:						
Fasting	137	142	132	132	137	139
100 cc	133	139	134	131	135	138
500 cc	128	138	129	132	135	136
1000 cc	133	139	131	134	136	137
Potassium in meq/l:						
Fasting	4.5	3.9	*	3.3	4.1	3.1
100 cc	4.0	4.3	3.1	2.8	4.0	3.6
500 cc	3.5	4.3	2.5	3.4	3.9	3.6
1000 cc	4.0	4.4	2.4	3.6	4.0	3.1
Chloride in meq/l:						
Fasting	122	107	104	98	99	110
100 cc	120	106	106	95	100	114
500 cc	114	109	107	102	102	110
1000 cc	122	108	109	102	98	114

* Hemolyzed serum.

Discussion

This investigation as carried out was to determine the effect of an intravenous infusion of polysal and/or 5% dextrose upon the blood level of sodium, potassium, chloride and sugar. The methods used have been presented and the results of the tests tabulated.

As might be expected, the results demonstrate an unpredictable variance in the effect upon the blood sugar level and a negligible change in the effect upon serum electrolytes.

POLYSAL

Patient	A	B	C	D	E	F	G	H	I	J
Infusion Time	3 Hrs.	2 Hrs.	3½ Hrs.	3 Hrs.	2 Hrs.	2½ Hrs.	4¼ Hrs.	3½ Hrs.	2 Hrs.	3 Hrs.
Sodium in meq/1:										
Fasting	139	135	134	137	140	131	132	129	135	129
100 cc	134	133	133	135	141	134	119	124	132	127
500 cc	135	120	135	133	143	135	123	124	134	124
1000 cc	138	131	135	135	140	131	122	120	135	124
Potassium in meq/1										
Fasting	3.6	2.9	4.3	3.8	3.0	3.8	4.5	5.1	4.0	4.6
100 cc	3.7	2.8	4.2	3.4	2.8	*	4.1	*	4.0	4.2
500 cc	3.6	2.8	4.1	3.5	2.6	4.0	4.4	5.2	3.3	4.3
1000 cc	3.7	3.2	4.1	3.6	2.9	4.1	4.5	5.1	3.5	4.2
Chloride in meq/1:										
Fasting	109	97	110	103	102	105	104	98	102	101
100 cc	108	96	106	102	104	107	102	99	100	100
500 cc	110	96	107	98	101	108	104	100	104	102
1000 cc	108	98	109	101	106	107	103	101	103	101

* Hemolyzed serum.

To be noted: The stability of all of these electrolytes irrelevant of amount and/or time of infusion.

It is evident that one cannot predict the level of blood sugar, even in approximation, while a patient is receiving a glucose solution intravenously. Furthermore, the time required for administration of the intravenous solution seems to be of little importance. We found both high and low values for the same length of infusion time. Each individual patient would seem to have his own relative glycogenesis rate.

The serum levels of sodium, potassium, and chloride are relatively stable during an infusion of an isotonic solution of these substances which indicates that a reasonably accurate prediction might be made in these determinations.

Summary

1. Serum sodium, potassium, and chloride may be determined with considerable accuracy using blood drawn during the process of an intravenous infusion of polysal or normal saline. The blood should be drawn from another vein than the one which is being used to infuse the solution.

2. Blood sugars vary during an intravenous infusion of glucose and should not be determined during that time.

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ON SHARPENING A MICROTOME KNIFE*

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Time was when every man was a skilled artisan to the extent that he knew how to keep a razor in condition. And usually the use of wood working tools gave him additional familiarity with the character of cutting edges and the means of creating and maintaining them. Now, thanks to modern technology, the up to date male can mow his whiskers without washing his face. The loss of ancient skills reflects itself in many ways, not the least of which is the nearly insoluble problem of obtaining a solid sharp knife for any purpose whatever. Evidence for this are the numerous substitutes derived from the safety razor that have been designed to replace the old-fashioned hand sharpened knife that could split an unsupported blond Nordic hair or in the hands of a Lisfranc could neatly detach a Guardman's leg in seconds.

The problem extends to microtome knives and has several origins both old and new. The notes that follow represent some of the things the writer learned in dim past days when as a medical student he cut sections evenings for his daily beans; no money—just beans. He had to find the nickel to ride the street cars somewhere else. The partner in this enterprise could never learn to sharpen the knife, but was good at nicking it, and eventually the pathologist became exasperated to the point that the team was fired. Misadventures with microtome knives have continued into recent years and finally have caused this essay on a very elementary matter which it is hoped will prove useful to those who instruct technologists in pathological technique.

First to be considered are materials. No need to sigh for the good old German knives. Their superiority was once real enough, but for many years now the domestic knives have left little to be desired. Stones are a source of difficulty.

The Belgian water stones, standard until World War II, an extremely fine "blue stone" and a companion yellow stone, still quite fine, but coarse enough to remove ordinary nicks, have disappeared without trace. Where they went, no one seems to know. An Army rumor has it that just before the War the government acquired all that were available and put them in warehouses where conscientious supply officers saw to it that they remained. Whatever the facts, the writer once journeyed to Oxford, and as a last resort, bought out of his own pocket a fine oil stone, presumably carborundum, from Mr. Cooper, ironmonger. This stone was much too small to be convenient but it ground rapidly and produced a sharp edge. Probably the best approximation is an oil stone designed for use with microtome knives which is produced by an American instrument maker. Actually, three stones of different grades are combined in the same mount. Water stones are on the market. The writer believes he has tried all of them and none are as fine as they should be. For reasons unknown many of them clog up with steel particles, something that the former stones did not do to a serious degree. One stone has a vile odor which adds substantially to the general reek of the laboratory.

* Received for publication January 2, 1958.

Concerning strops, there is nothing to regret. Some are charged with diamond dust and polish with great rapidity. As a consequence, they must be used sparingly, a point that will be returned to later.

What about mechanical knife sharpeners? The machines that are built around a glass plate or wheel seem quite popular. The writer once bought one in desperation. It worked. But obviously as a grinding tool, glass can only retain the finest abrasives and the action when nicks are to be removed is slow. And abrasives must be mixed and more important cleaned up afterward with great care. Fine abrasives are extremely tenacious and have a way of contaminating the premises. Accidental contact with precision machinery or optical surfaces can have undesirable consequences.

At least one machine on the market employs a rather coarse water stone. This is excellent for removing nicks, straightening up a sway-backed edge and, as will be noted later, for adjusting the bevel of the knife. Unfortunately, the machine gets used for routine honing, with the result that the knives disappear in clouds of steel dust—correction, go down the drain as a black powder.

Now to get on with the actual sharpening of a knife. In proposing a return to old fashioned hand methods for routine sharpening, the writer is not preaching a crusade against modernity but is offering a method that saves time and effort. A few minutes spent correctly on a knife saves hours in subsequent cutting—not to mention the salubrious effect that sections free from grooves, tears and wrinkles have on the disposition of the pathologist.

A. Equipment:

1. Hones: Perhaps a Belgian water stone can be found in a drawer devoted to junk. If not, then the best answer is probably the set of oil stones mentioned previously. The motor driven water hone is no liability. It saves the knives many a trip to the factory.

2. Strops: No problem.

3. Microscope: No problem, either; except, perhaps, getting the technicians to use it.

B. Method:

First to be considered is the structure of the knife and what one is trying to accomplish.

The knife is a wedge with a narrow bevel that leads to the cutting edge (Figure 1). To create an edge that meets the requirements of tissue work two distinct acts are involved; namely (1) grinding and (2) polishing. The distinction between the two has been recognized since remote times, both having many applications in the arts and trades.

Grinding consists in passing an object with pressure over a hard surface, with particles of an abrasive interposed, in our case the bevel of the knife and the hone. The particles, which are quite large in terms of microscopic dimensions, by a sort of crushing action, bite out particles of metal. The speed of grinding is determined by the particle size and the hardness of the abrasive. The particles themselves are crushed so that they lose their effectiveness and must be washed away (together with the particles of steel) with oil or water. The purpose of grinding (honing) is to give the edge the greatest possible continuity, that is to say, freedom from nicks. However, even the finest grinding agents leave an

infinity of nicks but these are small enough that they can be removed by polishing (stropping).

Polishing consists in rubbing an object over a soft but resistant surface in which is embedded a fine abrasive. Here the action is completely different from grinding. Metal is removed slowly, without the abrasive leaving a trace on the surface despite the fact that the particles are very definitely of finite size. The phenomenon of polish has always had a mysterious quality that has intrigued opticians since lenses were first made. At one time it was supposed to be dependent upon a flow of surface molecules. In any event, stropping, a polishing operation, removes the marks left by the hone and produces a perfectly smooth edge.

Honing: To return to Figure 1, the bevel has been created by honing with the knife back in place (Figure 2). It is essential that the back always be placed on the knife in the same direction. File marks on one end of the knife and back will identify which end is which. The reason for this precaution is that the bevel must lie flat on the hone (Figure 2) which it will not do if the angle given by the back is even slightly altered. The wear is never the same on both ends of the knife and back, hence the two must be kept matched. If the position of the back is reversed or if the back is not seated properly one will find himself grinding his heart out on the shoulder of the bevel (Figure 3A) along some portion of the edge.

Figure 1.

Figure 3.

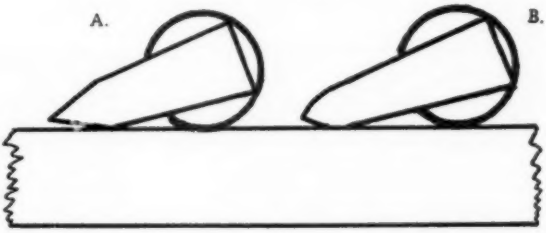


Figure 2.

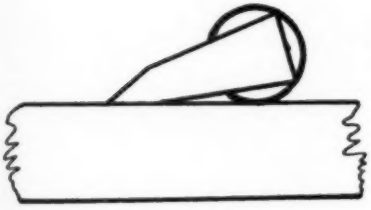
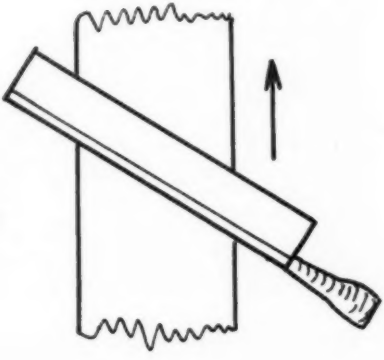


Figure 4.



The amount of metal removed by a fine stone with each stroke is extremely small. Some notion of the magnitude should be acquired by frequent examination of the knife under the low power of the microscope. This is important because it will save one from wasting his youth on nicks that are, for practical purposes, beyond the reach of the ordinary abrasive or on a bevel that was made too short by the manufacturer. The effect in the latter case is again that shown in Figure 3A. In the matter of nicks, Figure 5 is a photograph of a knife in a sad state. The edge is badly broken. The nicks are not too deep to be removed by hand but would require an excessive amount of time. This is an appropriate job for a machine. The picture tells more. The grooves at a right angle to the edge betray mechanical honing. Because the grooves persist to the edge, the knife was evidently used without stropping.

Figure 6 shows the effect of a badly adjusted knife back or, as was actually the case, a bevel that was too short in the first place. This was a new knife that was being honed for the first time. Note that the oblique lines produced by hand honing are not even close to the edge but are located on the shoulder of the bevel, again Figure 3A. To grind down to the edge would take an eternity so that the only solution is to return the knife to the manufacturer or readjust the bevel on a mechanical hone to make it correspond to the angle given by the knife-back. It is with knives like this one that Mephisto likes to break the backs of technicians who insist on working by rule of thumb and a time clock instead of by the microscope. For them, no tears.

Hand honing; the stroke. Many fancy motions are in vogue. These are as superfluous as the flourishes that a shoe-shine boy gives to his brushes and rag. The knife (handle and back in place) is simply pushed straight back and forth the length of the stone with the edge leading. The knife is held at an angle to the stone, Figure 4. With each reversal in direction the knife is rolled over on the back without losing contact with the stone and without altering the angle.* The appearance of the edge after a dozen or two strokes will be that in Figure 7. Note that the bevel is covered by grooves which extend to the edge which has a fine saw-like character. A relatively coarse stone was used to remove small nicks, of which only traces remain. The next step is honing on the finest stone available. This will change the appearance hardly at all, the grooves being only a little shallower and the saw-edge a little less apparent.

How much time has been "wasted"? Perhaps ten minutes.

Stropping: The knife is held at a right angle to the strop, not obliquely, and again the stroke is a straight one to and fro, this time the back leading, the edge trailing. A few deliberate strokes with pressure quickly polish out the marks left by the stone giving the appearances in Figure 8 which resulted from exactly a dozen strokes. The highlights along the edge indicate that a few more are needed. Note that the grooves in the bevel are more conspicuous than in the previous figure. This is due to the deeper grooves being unmasked, so to speak, by the polishing out of the finer ones. They are of no importance provided they do not reach the edge, and no effort should be wasted removing them.

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* To retard excessive wear of the middle of the knife most of the grinding is done on the ends. The proportion of knife-length and width of the stone is usually such that the middle is taken care of by the overlap.

Figure 5.

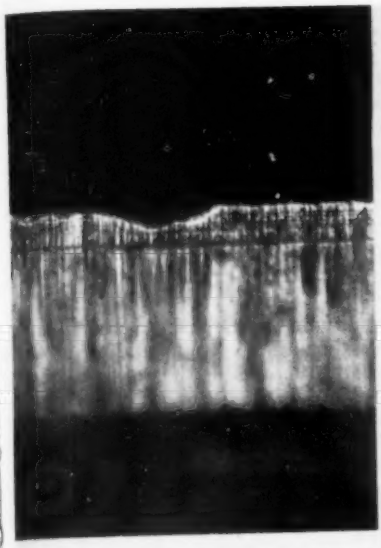
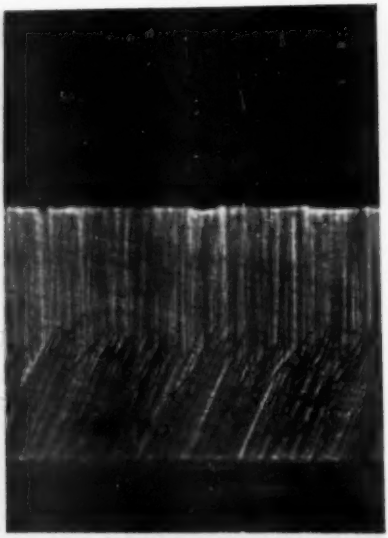


Figure 6.



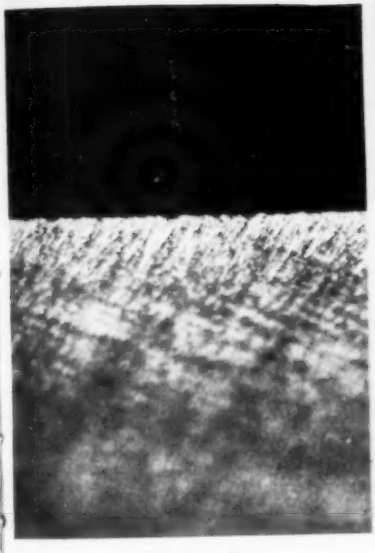


Figure 7.

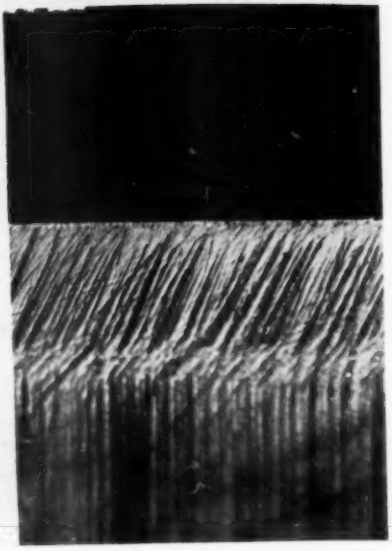


Figure 8.

The action of present day strops is extremely rapid due to incorporated diamond dust. This is reason for a word of caution. A dull knife, without gross nicks, can be restored by stropping. This is permissible a very few times, but when too long continued, the bevel becomes rounded so that when honing is finally undertaken, contact between the edge and the hone does not occur until a considerable amount of metal has been removed. (Fig. 3B). Only then does the work of sharpening the knife actually begin. It is probably this classic error of too much stropping and too infrequent honing, more than any other, that gives hand sharpening its bad name as a waster of time.

Care of hones: The hone as well as the knife suffers wear so that the surface is no longer flat. To ask anyone in our day to maintain his hones in proper condition is like urging him to go out in the woods and gather his own balsam as the early histologists were wont to do. Nevertheless, we shall describe the principle which requires the possession of two identical hones.

If a block of hard material is rubbed over another of the same dimension with an abrasive between them using a straight to and fro motion the upper one will gradually become concave and the lower one convex. By frequently reversing the position of the blocks the two can be brought to perfect flatness. It was in this manner that the architects of ancient Greece fitted the drums of their columns to perfection. This was hand work too—by the slaves of another republic. Again, unnamed monks about the 12th Century took advantage of the same physical phenomenon to grind spectacle lenses although they were usually more interested in producing spherical rather than flat surfaces. Anyhow, the hones should be kept flat whether by the method here described or by wrapping and mailing to the manufacturer for resurfacing.

Final note: A knife is an expendable item of equipment, not a permanent one. Eventually it wears out and must be thrown away or adapted to some meaner purpose. The life is quite long and in the course of it there will be deterioration that will require factory restoration and application of a new back. As the back wears the bevel of the knife is increased in width so that to eliminate even a small nick an excessive amount of metal must be removed. When the bevel reaches about 1.5 mm honing is no longer profitable and reshaping is in order. Deep nicks are removed by machine honing or, lacking one, confided to a knife sharpening service.

CONCLUSION: The maintenance of microtome knives requires the hand an eye of an informed technician, and there is no substitute. When the cutting quality begins to deteriorate the knife is honed briefly and stropped briefly, the progress of the work being controlled by the microscope to accomplish only what is necessary and to avoid labor that serves no purpose. When the back becomes worn and the bevel broadened or deep nicks have occurred, the knife is ready for factory reconditioning.

If these simple recommendations are followed the atmosphere of the tissue laboratory will always be as serene as a mill pond on a summer morning.

SERUM ALDOLASE DETERMINATION AS A ROUTINE LABORATORY PROCEDURE*

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Numerous observations indicate that serum aldolase is elevated in conditions associated with acute cellular destruction.[1] The clinical application of this observation has resulted in increasing demands for the determination of the concentration of this enzyme in hospital laboratories. The present study reviews various methods employed to assay the aldolase content of serum with proposed modifications in the form of a micro-technique which will permit routine use of the test.

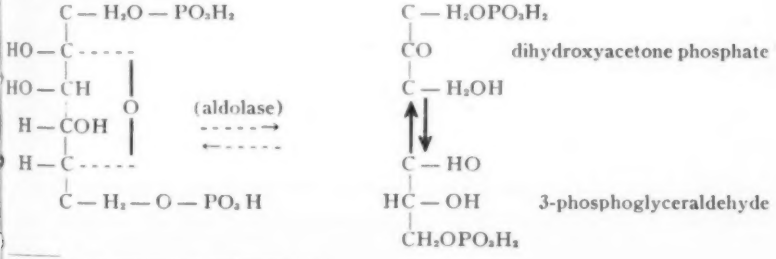
Significance of the Test

Aldolase is an enzyme that plays a crucial role in the chain of reactions involved in glycolysis. The concentration of aldolase in serum under normal conditions remains remarkably constant. There are much larger amounts of this enzyme in tissue cells, and elevations in the level of serum aldolase are thought to be due to acute, rather extensive destruction of tissue which might result in liberation of the intracellular enzyme at a rate greater than that which could be cleared from the circulation.[2] Such would be the case in extensive peripheral gangrene, large pulmonary infarcts, hemorrhagic pancreatitis and particularly acute hepatitis. In hemolytic anemia and leukemia the source is the destruction of the blood cells. The enzyme is also increased in diseases involving degenerative changes in muscle and brain tissue.

Chemical Principle of the Test

Fructose -1,6- diphosphate (henceforth referred to as HDP) is incubated with the buffered test sample and hydrazine to fix the triose phosphates formed.[3] At the end of the incubation period the reaction is stopped with trichloroacetic acid. An aliquot of the filtrate is then treated with alkali, followed by 2, 4- dinitrophenylhydrazine. On making the mixture alkaline again, a characteristic color appears with maximum absorption at 540 mu owing to the formation of a 2, 4- dinitrophenylhydrazine derivative of the triose. The intensity of the color is directly proportional to the enzyme concentration.

Chemical picture[4]



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Reagents—

I. Fructose -1,6- diphosphate: Commercial material from Schwarz Laboratories, Inc. (202 East 44th Street, New York 17, New York) is adequate in the assay method without further purification, but it contains too much inorganic phosphate to be used for setting up the curve.[3] The commercial products from Sigma or Nutritional Biochemical Company can be purified by the combined methods of Neuberg[5] and Dounce.[6]

A. Purification of Substrate.[5]

Add just enough trichloroacetic acid to dissolve the crude Barium salt. Add alcohol until precipitation no longer occurs. Collect the precipitate by filtration using a Buchner funnel. The precipitate is dissolved in cold water and reprecipitated by alcohol as before. Dry at room temperature and place in a desiccator. This Barium salt, if kept dry, is stable for several months and has been prepared commercially in dried ampules.

B. Conversion of the Barium salt to the Sodium salt.[6]

Dissolve 1.37 gm of the purified Barium salt in 15 ml of 1 N HCl. Add 5 ml of molar Na_2SO_4 solution slowly and with stirring. Centrifuge and test the supernate for completeness of precipitation by adding an extra drop of Na_2SO_4. If precipitation is complete, adjust the pH to 7.4 by adding 10% NaOH at first and finally 1 N NaOH. If the solution has a yellow color, it may be removed with Norit. If desired, the hexose diphosphate content of the substrate can be checked by the fructose method of Roe.[7] However, the activity of the enzyme is linear in substrate concentrations ranging from 0.0025 M to 0.01 M.[8] An approximate concentration of 0.025 M has proved reliable. Numerous observations have shown that conversion of 1.37 gm of Barium salt to the Sodium salt by the procedure given in this article will give from 0.075 M to 0.1 M concentrations of stock substrate.

The stock substrate is stable at least two weeks in refrigerator if 1 drop of toluol is added or it is better to keep it in the deep freeze. The stock substrate is diluted just before use with cold collidine buffer in a ratio of 1:3, or if "blank" values are too high for photoelectric measurement, the substrate is diluted 1:5.

II. Hydrazine: 0.56 M, pH 7.4 Merck's hydrazine sulfate is suitable. Add 30 ml of 10% NaOH to 7.3 gms hydrazine sulfate to facilitate solution. Then adjust the pH to 7.4 and dilute to 100 ml.

III. 0.1 M Collidine buffer:[9] (2-4-6 Trimethyl pyridine): Add 1.3 ml to approximately 80 ml of water. Adjust pH to 7.4 and dilute to 100 ml.

IV. 0.002 M Iodoacetate: Dissolve 1.35 gm iodoacetate (iodoethanoic acid) in approximately 80 ml adjust pH to 7.4 and dilute to 100 ml. This is a 0.2 M solution and must be diluted 1:100 for the test.[10]

V. 2,4-dinitrophenylhydrazine: 1 gm of 2,4-dinitrophenylhydrazine is dissolved in 1 liter of 2N HCl and the solution filtered. The reagent is stable if kept in the dark and cold.

VI. 0.75 N NaOH.

VII. 10% Trichloroacetic acid.

Preparation of Standard Curve[3]

Since the chromogen measured in the reaction is of unknown structure and pure samples of the triose phosphates are not commonly available, a standard curve relating % T to amount of HDP can be prepared either by using fresh homogenates of rat liver or commercial preparations of the enzyme in the crystalline form.

The enzyme can be obtained easily from the rat. The animal is decapitated with a large scissors. The liver is rapidly removed[11] and approximately 1 gram placed in an all glass homogenizer with 50 ml of cold water.[9] This crude homogenate will remain stable for several hours if kept cold.

A. The triose chromogen is obtained from 2 or 3 different concentrations of the homogenate by the same chemical test used for the unknowns except that 0.5 ml of 5% egg or bovine albumin is added to the buffer system instead of the water to give the dilute tissue extract the same enzyme protective protein as the serum test.

B. On separate aliquots of the trichloroacetic acid supernatants the alkali-labile P liberation is determined by measuring inorganic phosphate on two 1 ml aliquots, one of which is incubated with 1 ml of 2 N NaOH. (Prepare the NaOH fresh to avoid SiO_2.) Incubate for 20 minutes and neutralize with 1 ml of 2 N HCl.

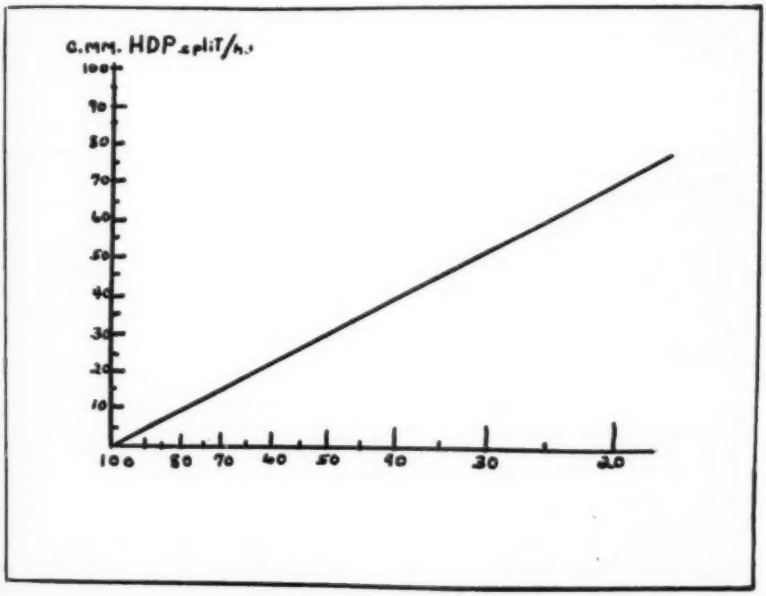

Fig. 1 illustrates a typical Standard Curve: % Transmission Serum Aldolase in Units per Ml. 1 unit of Aldolase = 1 c. mm. of H D P split at 38° C per hour.

C. The primary standard for the triose chromogen = the amounts of alkali-labile phosphorus. In order to convert galvanometer reading on 1 ml of trichloroacetic acid filtrate into this expression, transform the micrograms of alkali-labile P liberated per aliquot, measured into c. mm of HD P liberated per hour for the total reaction mixture by the following formula:

22.4 c. mm of H D P = 1 micromole of H D P
= 2 micromoles of triose phosphate
= 2 micromoles of alkali-labile P
= 62 gamma alkali-labile P

The galvanometer readings of the triose chromogen per 1 ml of aliquot are then plotted against c. m of H D P split calculated from the alkali-labile P liberated as outlined above. Once this standard curve has been prepared, it is unnecesary to restandardize the method with each set of determinations in these absolute terms, since the relationship between the O.D. of the triose chromogen and alkali-labile P liberation has been found to be constant and independent of small variations in different batches of reagents also independent of the source of enzyme assayed.[3]

As has been stated by Beck[12] the choice of a standard still remains a problem. The ideal standard, triose phosphate, is expensive, unavailable in pure form, and fairly unstable. However, the alkali labile calibration although cumbersome has been proven to be accurate.

Aldolase activity is also expressed as micro-grams of inorganic phosphate split from the dihydroxy-acetonephosphate per ml of serum per hour.[13]

Micro Method for Serum Analysis

Wash 100 lambda (0.1 ml) of serum into 0.8 ml of the buffer system. (This consists of 100 parts Collidine buffer, 25 parts Hydrazine, 25 parts Iodoacetate and 25 parts H_2O). Add 0.1 ml of HDP and incubate in a 38° C water bath for 60 minutes. Blanks for the above are also prepared and incubated but do not contain HDP. Add 1 ml of 10% trichloroacetic acid to all tubes. Add 0.1 ml HDP to blanks only. Centrifuge and to 1 ml of the filtrate add 1 ml 0.75 N NaOH. Let mixture stand at room temperature for 10 minutes. Add 1 ml of 2, 4 - dinitrophenylhydrazine. Place in 38° C water bath for 20 minutes. Then add 7 ml of 0.75 N NaOH. Read in 3 to 10 minutes on Spectrophotometer at 540 mu.

Comparison Studies of Methods

Recovery of aldolase by this method was far superior to the four other techniques studied. A normal value of 3 to 10 units (± 0.6 units/ml) was established which agrees with normal levels determined by the chemical tests elsewhere. Other methods studied were the original technique of Sibley and Lehninger,[3] of Dounce,[6] the modification of Dounce to the Sibley and Lehninger procedure[9] and the modification by Bruns.[13] Each of these methods gave relative results but recovery was often in error up to 20%. In the original method of Sibley,[3] sera occasionally failed to reveal any of the enzyme present while duplicate determinations of the same sample demonstrated the expected result.

This difficulty was also noted by Baker and Govan.[14] The notable changes to the original procedure are as follows:

1. The optimum pH with tris aminomethane buffer remained at 8.6 but with Collidine buffer the optimum activity occurs at pH 7.2.[16]

2. Collidine buffer gave more reproducible results[9] and fewer negative analyses.[14]

3. Iodoacetate was added to both the tris and collidine buffer studies to prevent other enzyme interference.[9, 15]

4. The substrate incubation with serum was increased from 30 minutes to 60 minutes.[13]

5. The substrate incubation for tissue assay was reduced to 15 minutes to minimize tendencies toward alkaline hydrolysis of triose phosphate and non-linearity of enzyme activity with time.[12]

6. The chromogen development reaction was increased to 20 minutes to permit osazone formation to approach completion and yielding triose phosphate chromogens with equal color values. This also increases the sensitivity and accuracy of the analyses.

Clinical Studies

Elevation of the serum enzyme level of aldolase has been suggested as a diagnostic aid in many unrelated diseases. The results of investigation into various diseases are shown in Table I.

TABLE I—SERUM ALDOLASE SURVEY

CATEGORY	No. of Patients	No. of Determinations	Aldolase Av. Units	Aldolase Range	Average Transaminase
Normal	50	75	6.9	3–10	
Muscle Injury	1	3	30	29–31	168
Fever Unknown Origin	3	5	10	6–14	
Infectious Hepatitis[23]	9	31	42	14–94	61
Pancreatitis[24]	3	4	25	10–30	
Multiple Sclerosis	1	3	5	4–11	
Carcinoma[17, 21, 22]	5	21	9	3–20	20
Myocardial Infarcts	6	10	8	4–64	79
Muscular Dystrophy[18, 19, 20]					
Muscular Dystrophy, Age 8	1	5	51	40–51	
Muscular Dystrophy, Age 13	1	6	43	35–44	80
Muscular Dystrophy, Age 14	1	3	31	30–33	
Muscular Dystrophy, Age 34	1	1	57	57	50
Muscular Dystrophy, Age 40	1	7	13	12–14	30
Muscular Dystrophy, Age 45	2	5	14	11–15	24
Muscular Dystrophy, Terminal Specimen	1	4	2	0–4	
Cirrhosis	4	12	14	8–22	48
Thorazine Jaundice	3	8	9	8–16	50
Obstructive Jaundice	3	7	20	11–26	
Leukemia	4	10	19	6–31	
Lymphoma	2	5	16	9–24	
First and Second Degree Burns					
First and Second Degree Burns, 2 hours	1	5	20	17–21	46
First and Second Degree Burns, 24 hours	1	4	43	36–45	74
First and Second Degree Burns, 48 hours	1	7	120	90–134	140
First and Second Degree Burns, 72 hours	1	8	100	84–111	98
First and Second Degree Burns, 10 days	1	5	10	6–14	78
Spinal Fluid from Encephalitis	1	3	0	0	

Discussion

Normal values are remarkably constant with controlled technique. Increases in aldolase concentration are generally found in proportion to acute cellular destruction. In connection with the problem of correlating serum enzyme activity in essential metabolic, sequences are present in many tissues and any effect on the integrity of these tissues may cause the release of the enzyme into the blood.

Summary

The procedures for the macro and micro determination of serum aldolase have been evaluated and a standard micro method described in detail. Techniques helpful in assuring accuracy and comparable data on results are also included. Examples of serum levels in various diseases are tabulated.

Acknowledgments

The kind assistance of Dr. G. O. Broun, Director of the Department of Internal Medicine, Firmin Desloge Hospital, St. Louis, Mo., at whose suggestion this work was done, is gratefully acknowledged. Appreciation is also expressed to Dr. Bernadette Bocklage, Assistant Professor in Biochemistry at St. Louis University, for her counsel and co-operation.

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A METHOD FOR EMBEDDING AND STAINING BONE MARROW FRAGMENTS*

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Introduction

It is well known that the microscopic study of the bone marrow is essential in the diagnosis and in following the response to specific therapy in hematological disorders. In this laboratory we have been using a method of processing and staining of the material obtained from bone marrow aspirations with uniformly good results and considerable simplification of the technical procedure. In many instances the results of bone marrow aspiration have been superior to those obtained from surgical biopsies. It is the purpose of this paper to report our method of embedding and staining fragments obtained from bone marrow aspirations.

Technique

In the preparation of bone marrow obtained from aspiration the procedure generally followed consists of routine fixation, followed by wrapping of fragments in lens paper. In this form the material is carried through the usual processing cycle up to the point of embedding. At this stage the technologist has the tedious chore of teasing the minute fragments from the lens paper and embedding them into a compact mass in the paraffin block. Even with the greatest care in embedding, the fragments tend to be arranged loosely and at varying levels, so that the finished slides show the bone marrow fragments almost invariably in a scattered pattern, rather than in a concentrated area, consequently it is necessary to cut the block at several levels in order to insure examination of several fragments.

Fixation and embedding. To avoid this difficulty we proceed as follows:

1. Immediately upon completing the aspiration, the liquid mixture of blood and bone marrow fragments is ejected onto a piece of filter paper, to absorb a major portion of the blood.

2. The fragments of bone marrow are carefully scraped from the filter paper with a scalpel and are transferred to a small piece of paper towel where they are arranged in a compact mass, with the aid of the blood clot. Any material remaining in the syringe barrel is removed and added.

3. The paper with the clot containing the bone marrow fragments is then placed in a liberal amount of Zenker's Solution acidified with 5% of acetic acid.

4. After a minimum of twelve hours of fixation the paper towel with the attached clot is wrapped in a small piece of lens paper. This is placed in a tissue capsule, washed in running tap water for a minimum of six hours and processed in the routine manner. The bone marrow fragments in the solid clot adhere to the paper towel through all the procedures from fixation to embedding.

* SPF Award, 3rd Histology, 1957. Read before the 25th Annual Convention, ASMT, Chicago, Illinois, June 1957.

5. To embed, the technologist needs only to lift the single clot of blood containing the fragments from the paper and to embed it in a paraffin block. The sections are cut as thin as possible, preferably not over 3 to 4 micra.

Preparation of stain

In most laboratories bone marrow sections are stained with the usual Hematoxylin-Eosin method. In special cases the technologist is requested to use the Giemsa stain. The results of this procedure vary considerably, depending on the technique used. To obtain more uniform results the following method has been developed. This is based on the use of the original Strumia Universal Blood Stain as published in the JOURNAL OF LABORATORY AND CLINICAL MEDICINE in 1936. The stain combines the properties of the Giemsa method with those of the May-Gruenwald in one solution and is prepared as follows:

Finely ground Azure II-eosin mixture (Giemsa) 1.3 gm. are suspended in 80 ml of pure glycerine and allowed to dissolve, shaking occasionally, for two or three days. This suspension is then heated in the water bath at 60° C. for two hours, mixing occasionally. After cooling the dye and glycerine is added to a mixture of methyl alcohol, 290 ml and acetone C.P., 290 ml.

Prepare separately the following solution: methylene blue-eosin powder (May-Gruenwald) .15 gm., methyl alcohol 170 ml; acetone C.P. 170 ml. The dye will dissolve in several days, with occasional shaking. When entirely dissolved, it is added to the Azure II-eosin solution and mixed. Occasionally, not all of the Azure II-eosin dissolves; it is better to allow the precipitate to remain in the bottom of the bottle as it will usually dissolve in time. If not, it does not interfere with the staining property of the solution. The completed stain should be allowed to stand for one month before use; it keeps well for at least one and one half years.

Staining Technique

1. Deparaffinize as usual and carry through to 95% alcohol.
2. Place for 15 minutes in a solution of 0.5% of iodine in 95% alcohol.
3. Carry through descending alcohols to water.
4. Place for 15 minutes in a solution of 0.5% of sodium thiosulfate.
5. Rinse in water for 1 minute.
6. Place overnight in a 1:30 dilution of Strumia Universal Blood Stain in distilled water.
7. Differentiate in a solution of 55 ml of 95% alcohol, 1 drop of 5% sodium carbonate and 1 drop of glacial acetic acid, using a medicine dropper. The stock stain solution when newly prepared tends to be alkaline and requires a slightly longer time for differentiation; the optimum is easily determined by low power microscopic observation. The differentiation is carried out until a point is reached when the nuclei are distinct and the chromatic detail can be seen. In general this is accomplished after a few dips.
8. Pass rapidly 3 times through absolute alcohol.
9. Pass through xyol, using 2 changes with 5 minutes of immersion in each.
10. Mount.

Results:

The staining characteristics of bone marrow section when prepared by this method are similar to those of a stained smear of bone marrow or peripheral blood.

This method for embedding and staining of aspirated bone marrow fragments results in a section with closely packed fragments of bone marrow, sharply and rather brilliantly stained, permitting ready differentiation of cellular elements. The procedure is simple and has been used routinely in this laboratory with consistently good results.

Summary

A method is presented for the embedding of fragments obtained from bone marrow aspirations, and the staining of paraffin sections using the Strumia Universal Blood Stain.

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A STUDY OF METHODS FOR DETERMINING SENSITIVITY OF BACTERIA TO ANTIOBIOTICS AND THE FACTORS INFLUENCING DIFFERENCES IN RESULTS*

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Now that the use of antibiotics in the treatment of infectious diseases is a well-established procedure, the ever-increasing number of new antimicrobial agents available has made the in vitro sensitivity testing of bacterial strains isolated from patients of great value to the clinician in selecting the most effective antibiotic for treatment. Even though this sensitivity testing has become routine in most clinical bacteriology laboratories, there are still problems concerning its conduct and interpretation.

The purpose of this study is: (1) to explore some factors responsible for variations in results of the more commonly employed procedures for sensitivity testing. It is hoped that such a study could lead to some standardization, and (2) to work out a more rapid method for the completion of in vitro sensitivity tests. If the time interval between the collection of the specimen and the reporting of some degree of susceptibility or resistance of the bacteria involved could be shortened even by a few hours, the clinician would be aided greatly in selecting the drug of choice for treatment.

There are two general types of sensitivity tests—the tube serial dilution and the agar diffusion methods. Each has its advantages and drawbacks, and laboratories must decide for themselves under what circumstances the type of test they will use.

The tube serial dilution technique is quantitative, giving the least concentration of antibiotic which inhibits growth. Its drawback is that it requires a great deal of equipment, large amounts of material, and much time.

The agar diffusion method is much simpler, more economical to perform, and requires less equipment, but has the drawback of results that are only qualitative. Its other, more objectionable feature is the involvement of too many variables which may lead to errors in interpretation. The rate of diffusion of antibiotics into solid medium varies with different antibiotics. Therefore, the differences in size of the zone of inhibition of growth around disks of two antibiotics are not related to differences in the susceptibility of the bacterium to the drugs. The size of zone of inhibition depends upon the solubility, and the rate of diffusion of the antibiotic, and the rate at which the bacteria grow. The edge of the zone of inhibition represents the distance that the antibiotic has traveled in a concentration sufficient to inhibit growth before the bacteria have grown sufficiently to be visible. In both testing methods, results are usually recorded after 18-24 hours incubation.

Bondi[1] used blood agar (veal infusion agar, 2.0% tryptose, and 10.0% defibrinated horse blood); Scott[2] used blood agar, eosin methylene blue agar, or chocolate agar; Hoyt and Goolden[3] used blood agar or eosin methylene blue agar, depending on the test organism. Neter, Murdock,

* 2nd Registry Award 1957. Read before the 25th Annual (ASMT) Convention, Chicago, Illinois, June, 1957.

and Kunz[4] have shown conclusively that the composition of culture media can have a profound influence on the degree of inhibition of growth by an antibiotic. Moreover, they state that while the effectiveness of a given antibiotic may be increased on one medium with a certain organism, the reverse may be true with another bacterium. There may be considerable variation from one antibiotic to another on the same medium depending on the bacterium being tested. Flanigan,[5] working with streptomycin, has shown that agar in media decreases its antibacterial activity. This cannot be explained as due to poor diffusibility alone. By adding small, but varying, amounts of agar to the tube broth serial dilution test, he has shown that decrease in activity of streptomycin was directly in proportion to the increase in agar concentration.

Another factor influencing the final results of in vitro sensitivity testing is the pH of the medium. Eagle[6] states that the antibacterial activity of penicillin against *Staphylococcus aureus* decreases with increase of pH in the range 5.7 to 7.7, whereas the reverse is true with streptomycin. He cites the fact, also, that other antibiotics have well-defined optimum pH ranges. Waksman, Bugie and Schatz[7] found that an increase from pH 7.0 to pH 9.0 greatly increased the activity of streptomycin. Foster and Woodruff[8] noted that in agar plates at pH 5.5 penicillin was three times more effective against bacteria than at pH 7.0 but that this effect on penicillin could not be demonstrated in broth. Abraham and Duthie,[9] however, found the reverse to be true: that the activity of penicillin in broth was increased, but there was no change in agar.

Welch,[10] in evaluating sensitivity methods, stressed the fact that many variables in performance of the tests may lead to incorrect interpretation, and that there is great need for standardization.

Materials and Methods

The broth dilution and plate diffusion tests reported in this study have been modified in four ways: (1) composition of media, (2) size of inoculum, (3) age of the cultures used as test organisms, and (4) length of incubation of the sensitivity tests.

ANTIBIOTICS: Difco sensitivity disks were used in all of the agar plate diffusion tests except the neomycin and albamycin (novobiocin).* The concentrations used are indicated in the Tables of results. For the tube dilution technique, vials containing 20 mgs. each, of sterile buffered antibiotics were used for preparation of stock solutions. By adding 20 ml. of sterile distilled water to a vial we had a stock solution of 1,000 mcg. per ml. for all antibiotics except penicillin. In the case of penicillin the concentration was 1,000 units per ml.

The stock solution was stored in the deep freezer at minus 5° C. until used, but for no longer than eight weeks.

MEDIA: In the tube dilution series of tests the following nine media were used:

(1) Tryptose broth (2.0% tryptose, 0.5% NaCl)
(2) Tryptose-dextrose broth (2.0% tryptose, 0.5% NaCl, 1.0% dextrose)

* Furnished by the Upjohn Company.
** Antibiotics for serial dilution tests obtained through the courtesy of Dr. Frederick C. Fink of Charles Pfizer and Company, Inc.

(3) Tryptose-blood broth (2.0% tryptose, 0.5% NaCl, 5.0% citrated human blood)
(4) Nutrient broth (0.3% beef extract, 0.5% bacto-peptone, 0.5% NaCl)
(5) Nutrient-dextrose broth (0.3% beef extract, 0.5% bacto-peptone, 0.5% NaCl, 1.0% dextrose)
(6) Nutrient-blood broth (0.3% beef extract, 0.5% bacto-peptone, 0.5% NaCl, 5.0% citrated human blood)
(7) Proteose peptone #3 broth (2.0% proteose peptone #3, 0.5% NaCl)
(8) Proteose peptone #3-dextrose broth (2.0% proteose peptone #3, 0.5% NaCl, 1.0% dextrose)
(9) Proteose peptone #3-blood broth (2.0% proteose peptone #3, 0.5% NaCl 5.0% citrated human blood)

In the agar plate diffusion method the same nine media were used by adding 1.5% agar in each case. All the media were titrated to a pH of 7.2.

CULTURES: The 40 cultures used in this study were isolated from specimens sent to the City of Memphis hospital bacteriology laboratory. All cultures used were from patients whose history revealed no antibiotic therapy during the current illness. Following are the cultures used and the number of strains of each: *Micrococcus pyogenes var. aureus*, 8; *Streptococcus pyogenes*, 8; *Diplococcus pneumoniae*, 2; *Streptococcus viridans*, 1; *Escherichia coli*, 8; *Corynebacterium diphtheriae*, 3; *Neisseria meningitides*, 2; *Hemophilis influenzae*, 1; *Proteus*, 4; *Salmonella typhosa*, 2; and *Shigella flexneri*, 1.

Five of these cultures—*M. pyogenes var, aureus, E. coli, Proteus, Salm. typhosa, and Sh. flexneri*—were maintained on nutrient agar and transferred at two week intervals. The other six—*Str. pyogenes, D. pneumoniae, Str. viridans, C. diphtheriae, N. meningitides*, and *H. influenzae*—were grown on nutrient agar with 5.0% blood added and transferred at 4-day intervals.

The cultures grown on nutrient agar after 24 hours incubation at 37° C. were stored in the refrigerator at 6° C. The cultures on the nutrient agar with blood added were maintained at 37° C., and, therefore, transferred more frequently.

TUBE DILUTION TECHNIQUE: The stock solution was removed from the deep freezer as needed and diluted with appropriate broth to a concentration of 200 mcg. or units per ml. The number of tubes used in the serial dilution test varied from 10 to 15, depending on the anticipated end point or sensitivity of the bacterium being tested.

Into tubes 2 through 10 (or 15) was pipetted 0.5 ml. of broth. Into the first and second tubes was pipetted 0.5 ml. of the antibiotic of 200 mcg. or unit contration. The contents of the second tube were mixed thoroughly and 0.5 ml. of it transferred into the third tube. This same procedure was followed down through the next-to-the last tube. From this next-to-the-last tube 0.5 ml. was discarded. The last tube, as the culture control, contained no antibiotic. To all tubes in the test, including the control tube, was added a 0.5 ml. portion of the broth-diluted culture.

In this setup we had a twofold descending dilution from 100 to 0.39 mcgs. or units per ml. After incubation at 37° C., the test was checked for cloudiness as evidence of growth. The lowest concentration of an antibiotic that prevented growth of the test organism was reported.

AGAR DIFFUSION TECHNIQUE: The plates were inoculated in such a manner as to have uniform growth over the surface. To prevent contamination the antibiotic disks were placed on the plates with forceps dipped in alcohol and flamed. The disks were applied immediately after inoculation, since it is very important that no growth of the organism take place before the disks are placed on the plate. To avoid overcrowding, the disks were placed at least 4 cm. apart. After incubation, the plates were examined for zones of inhibition of growth.

COMPOSITION OF MEDIA: In order to keep at a minimum the variation in different lots of culture media, all broth and agar were prepared by one investigator. Care was taken to have the volume of agar in each petri plate the same, since differences in depths of agar would have introduced another source of errior in making the comparison of the sizes of zones of inhibition of growth around the antibiotic disks.

Both the agar diffusion and broth serial dilution tests were used for the comparison of 40 cultures on nine different media. These 40 cultures represented 14 different species of bacteria. Just prior to testing, each of the five cultures maintained on nutrient agar was grown for 18 hours at 37° C. on tryptose broth. The six cultures maintained on nutrient blood agar—*Str. pyogenes, Str. viridans, C. diphtheriae, N. meningitides, H. influenzae,* and *D. pneumoniae*—were grown on tryptose blood broth.

The plates, each one containing exactly 15 ml. of agar, were poured and allowed to remain at room temperature for 18 to 24 hours before being used. This not only permitted a check on sterility but also allowed time for the surface of the plates to be uniformly dry. The plates were inoculated with 0.1 ml. of the 18-hour broth culture, and the antibiotic disks were immediately applied aseptically. For the broth serial dilution, the cultures were diluted 1:100 and added in equal amounts to the tubes of serially diluted antibiotics.

Both the agar diffusion and the broth dilution tests were incubated at 37° C. for 18 hours. At the end of this period, the zones of inhibition of growth around the antibiotic disks were measured, and the least concentrations of the antibiotics in broth completely inhibiting growth were noted.

SIZE OF INOCULUM: The cultures for this experiment were grown in the same manner as for the previous experiment. At the end of the 18-hour incubation period, the cultures were centrifuged and the sediment re-suspension in buffered saline. Using a barium chloride nephelometer, the suspension was matched to the #5 standard (approximately 1,500,000,000 bacteria per ml.). This suspension was designated as "undiluted." Dilutions of 1:100 and 1:10,000 were made. Using tryptose blood agar for the agar disk diffusion and tryptose blood broth for the broth serial dilution, sensitivity tests were run simultaneously on the three sizes of inocula. The technique for the tests was the same as used in the preceding experiment.

AGE OF CULTURE: In this series of experiments the size of the inoculum (1:100 dilution) and the type of media used (tryptose-blood agar and broth) were kept constant, and the ages of the cultures were varied. We chose to use 6-, 18-, 24- and 48-hour-old cultures. The results of these sensitivity tests were observed after 18 hours incubation at 37° C.

LENGTH OF INCUBATION TIME OF SENSITIVITY TESTS: Observations of the results of the preceding experiment both for agar disk diffusion and broth serial dilution tests were carried one step further for the 18-hour-old cultures. The zones of inhibition of growth on the agar plates were measured, and the least quantity of antibiotic completely inhibiting growth in the broth series was noted. These observations were made at the end of 6, 18, 24, and 48 hours, respectively.

Results

COMPOSITION OF MEDIA: Analysis of results showed that there is a close, though not absolute, correlation between results of both the agar disk diffusion and the broth dilution methods. The influence of the composition of culture media on inhibition of growth by antibiotics is summarized in Tables 1, 2 and 3.

It was noted that with any one bacterium, results varied, depending on which medium was used. We found that one medium enhanced the activity of one antibiotic, while the antibacterial action of another antibiotic was favored by another, different medium. To illustrate this: (Table 1) Aureomycin gave the largest zones of growth inhibition of *M. pyogenes var. aureus* on tryptose agar, while streptomycin was more active against the same organism on proteose peptone #3 agar. When *E. coli* was our test organism (Table 2), aureomycin and streptomycin

TABLE 1

DIAMETER OF GROWTH INHIBITION ZONES* BY SIX ANTIBIOTICS USING CULTURE MEDIA OF DIFFERENT COMPOSITIONS

CULTURE MEDIA	Aureomycin (10 mcg.)	Chloromycetin (10 mcg.)	Penicillin (10 u.)	Streptomycin (10 mcg.)	Terramycin (10 mcg.)	Tetracycline (10 mcg.)
Tryptose	22.62	20.77	28.50	8.25	15.63	21.00
Nutrient	22.00	16.77	19.62	12.00	17.87	20.25
Proteose Peptone # 3	21.62	17.81	24.87	16.00	18.00	22.50
Tryptose + dextrose	22.25	19.37	24.37	7.27	16.50	22.50
Nutrient + dextrose	22.25	16.00	24.25	11.62	19.12	22.12
Proteose peptone #3 + dextrose	19.50	17.22	21.75	14.00	15.75	20.25
Tryptose + Blood	19.12	14.57	24.25	6.62	16.37	17.88
Nutrient + Blood	18.62	8.71	20.00	11.07	16.37	17.62
Proteose peptone #3 + Blood	15.13	13.12	15.75	10.20	12.75	13.12

* Diameter of Zones (mm) Average for 8 strains of M. pyogenes var. aureus

TABLE 2

DIAMETER OF GROWTH INHIBITION ZONES* BY SIX ANTIBIOTICS USING CULTURE MEDIA OF DIFFERENT COMPOSITION

CULTURE MEDIA	Albamycin (100 mcg.)	Aureomycin (10 mcg.)	Chloromycetin (10 mcg.)	Streptomycin (10 mcg.)	Terramycin (10 mcg.)	Tetracycline (10 mcg.)
Tryptose	1.21	1.83	9.83	9.16	3.83	2.16
Nutrient	3.50	5.00	14.50	11.50	2.33	7.50
Protease peptone # 3	1.20	1.66	14.00	8.66	2.66	4.66
Tryptose-dextrose	5.33	9.83	12.50	8.50	9.60	9.66
Nutrient-dextrose	1.66	17.00	8.83	5.00	9.83	11.50
Protease peptone #3-dextrose	2.55	11.5	16.00	8.50	2.55	8.16
Tryptose-blood	1.33	2.00	5.66	4.33	2.83	2.50
Nutrient-blood	0	5.50	7.83	5.50	1.93	7.50
Protease peptone #3-blood	1.33	3.00	12.50	7.00	2.00	3.00

* Diameter of zones (mm) Average of 8 strains of E. Coli

gave the largest zones with nutrient agar. When *M. pyogenes var. aureus* was tested, the addition of blood to the broth or agar decreased the activity of all antibiotics tested. In the tests using *E. coli*, the decrease in activity of the antibiotics when blood was added to the media was noted in all cases except with aureomycin. With both *M. pyogenes var. aureus* and *E. coli*, the peptone without blood or dextrose seemed to be the overall choice of media.

Salm. typhosa, Sh. flexneri, and *Proteus* all fell into essentially the same pattern as *E. coli* on the various media tested.

When testing the more fastidious organisms—*H. influenzae, Str. pyogenes, D. pneumoniae, Str. viridans, C. diptheriae,* and *N. meningitides*—only the three media containing blood were used. Table 3 shows the results of the sensitivity tests on *N. meningitides and Str. pyogenes,* which are representative of this group. Here again, there is no one medium that would be equally satisfactory for all bacteria with every antibiotic.

To point out a few of the inconsistencies, nutrient blood agar gave best results when testing the sensitivity of *N. meningitides* to aureomycin, but proteose peptone #3 blood agar gave the largest zone for this organism with tetracycline. Chloromycetin was most active against *Str. pyogenes* on tryptose blood agar, but terramycin was most active on nutrient blood agar.

TABLE 3

DIAMETER OF GROWTH INHIBITION ZONES BY SIX ANTIBIOTICS USING CULTURE MEDIA OF DIFFERENT COMPOSITIONS

CULTURE MEDIA	Aureomycin (10 mcg.)	Chloramycetin (10 mcg.)	Penicillin (1 u.)	Streptomycin (10 mcg.)	Terramycin (10 mcg.)	Tetracycline (10 mcg.)
*						
Tryptose-blood	16.00	20.00	0	15.00	12.00	20.00
Nutrient-blood	40.00	15.00	36.00	20.00	24.00	14.00
Protose-peptone #3 blood	20.00	22.00	40.00	12.00	24.00	28.00
**						
Tryptose-blood	21.00	18.00	24.00	0	24	24.50
Nutrient-blood	8.00	16.00	16.50	0	26.50	27.5
Protose-peptone #3 blood	23.00	16.50	17.00	5.50	18.50	22.5

* diameter of zone (mm) Average of 2 strains N. meningitides

** diameter of zone (mm) Average of 8 strains Str. pyogenes

SIZE OF INOCULUM: Figures 1 and 2 show the results obtained using the three different sizes of inocula with eight strains of *M. pyogenes var. aureus*. With each antibiotic the concentration required to inhibit growth increased as the size of inoculum increased. The same results were obtained with all organisms tested. The increase in the amount of inhibiting concentration was greater in the serial dilution tube sensitivity test in proportion to the inoculum than was the amount needed for inhibition in the agar diffusion tests.

AGE OF CULTURE: Figure 3 shows the definite effect of age of the culture on the results of sensitivity tests. With both types of tests, the resistance of all cultures to all antibiotics increased with the increase in age of the culture.

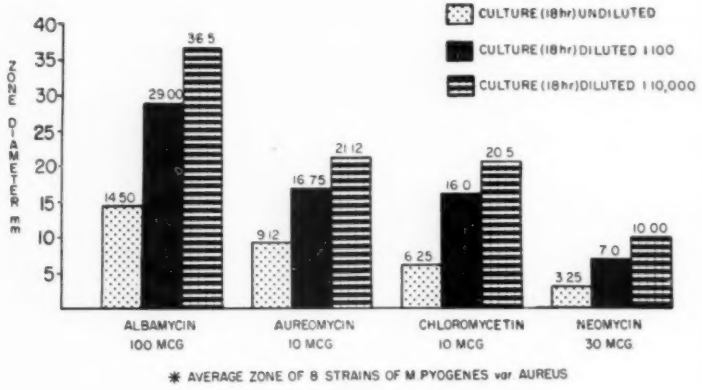
LENGTH OF INCUBATION TIME OF SENSITIVITY TEST: The curve in Figure 4 shows a striking resemblance to the previous one. The earlier a sensitivity test was observed after visible growth, the more susceptible to the antibiotic the organism was likely to be. *H. influenzae* and *N. meningitides* were the only cultures that did not grow sufficiently within six hours to allow the agar disk diffusion plates to be read.

Discussion

These experiments have shown that composition of culture media influenced the results of antibiotic sensitivity tests. They have also demonstrated that the younger the culture used and the shorter the incubation period of the test, the more sensitive the organism was, regardless of the antibiotic used.

DIAMETER OF GROWTH INHIBITION ZONES*

DISK PLATE DIFFUSION METHOD WITH VARIED INOCULATION SIZE

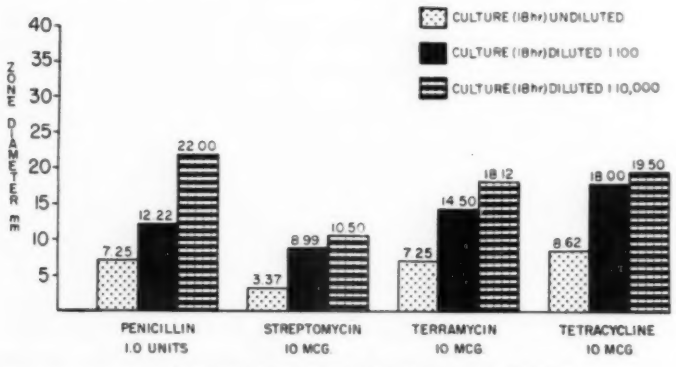


* AVERAGE ZONE OF 8 STRAINS OF M. PYOGENES var. AUREUS

Figure 1

DIAMETER OF GROWTH INHIBITION ZONES*

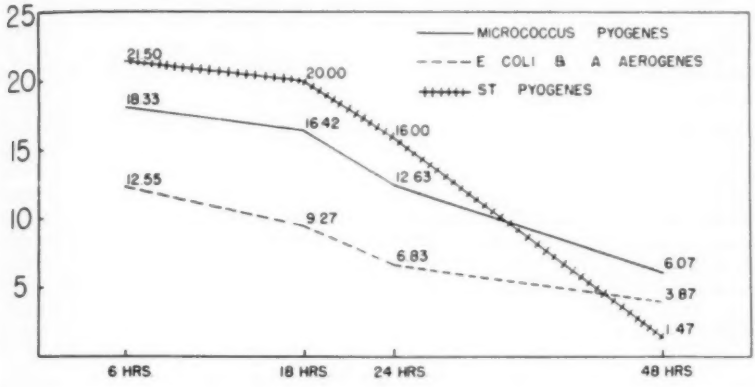
DISK PLATE DIFFUSION METHOD WITH VARIED INOCULATION SIZE



* AVERAGE ZONE OF 8 STRAINS OF M. PYOGENES var. AUREUS

Figure 2

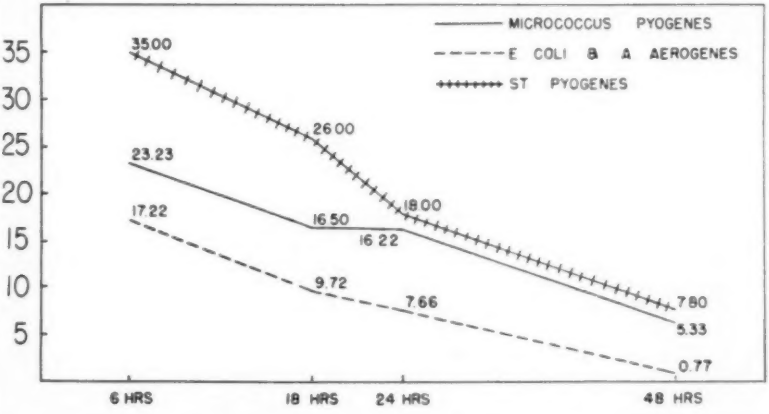
DIAMETER OF GROWTH INHIBITION ZONES* OBTAINED IN DISK PLATE DIFFUSION METHOD AS AFFECTED BY CULTURE AGE



* ZONE IN mm. — AVERAGE OF 8 STRAINS WITH 6 ANTIBIOTICS

Figure 3

DIAMETER OF GROWTH INHIBITION ZONES* OBTAINED IN DISK PLATE DIFFUSION METHOD AS AFFECTED BY TEST INCUBATION TIME



* ZONE IN mm. — AVERAGE OF 8 STRAINS WITH 6 ANTIBIOTICS

Figure 4

No one medium afforded conditions most favorable for all bacteria with all antibiotics. We recommended the selection of one medium that would, as nearly as possible, permit optimal growth of all pathogens. For this medium, we have selected and are now using nutrient agar with 1.0% tryptose and 5.0% human blood added. Even though blood added to culture media decreased the activity of most antibiotics, this was more than offset by the decreases in time needed for growth of the test culture and in time necessary for incubation of the sensitivity test. In addition, the use of the one medium for all tests brings about the much-desired standardization of the test.

In the study of sensitivity tests with varying incubation periods, it was noted that most species of bacteria had grown sufficiently to be reported within six hours time. Comparison showed that sensitivity test plates which were heavily inoculated could be read after six hours incubation period, while other plates that were inoculated with a lighter suspension could not be read before the usual time period of 18 to 24 hours. At the present time 19 strains of bacteria have been compared with encouraging results. By using the enriched blood agar, the tests using heavy inoculation with 6-hour incubation periods gave zones of inhibition of growth of approximately the same diameter as the standard sensitivity test after the 18 to 24 hours incubation required for its completion. *N. meningitides* and *H. influenzae* were the only organisms encountered that failed to produce visible growth within six hours.

Summary

A study has been made and reported of the effect on the results of the routine antibiotic sensitivity tests of these four important factors: (1) composition of culture media, (2) size of inoculum, (3) age of culture, and (4) length of incubation period of test. A modification of the routine test has been proposed that shortens the time consumed in its performance by 12 to 18 hours.

Acknowledgement

The author wishes to express her appreciation
to
Dr. I. D. Michelson
for his interest and invaluable suggestions during this study.

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CORRECTION

January-February 1958 issue. In the article entitled, "A Micro Method for Blood Sugar Using Anthrone," by Mary A. Nugent and David G. Fleming. Second paragraph, line 4, should read, "dextran,[2] *irulin*,[3] ----------," Reference #3 should read: "White, R., and Samson, F.: Determination of *inulin* in plasma and urine by use of anthrone, ----------"

Classified Ads Continued on Page XXXVIII—Advertising Section

IMPROVING THE ACCURACY OF EOSINOPHIL ENUMERATION IN ROUTINE DIFFERENTIAL LEUKOCYTE COUNTS*

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A slightly or moderately elevated eosinophil percentage in an otherwise normal differential leukocyte count is a frequent occurrence. Depending on the clinical picture, eosinophilia may direct the clinician's attention to the possibility of parasitic infestation, allergy, Hodgkins disease, periarteritis nodosa, or a number of other conditions. In most cases a report of slight or moderate eosinophilia will be followed by requests for repeat blood counts, chamber eosinophil counts, stool examinations for parasites, and possibly other tests. Yet there is no doubt that many reports of eosinophiles in the range of 5 to 15 or 20% are inaccurate and do not really represent eosinophilia. We wish to point out the reason for this inaccuracy and a simple method for overcoming it.

In most laboratories the routine differential count is based upon observation of 100 cells under the oil-immersion lens, while 200 cells or more are counted if there is leukocytosis or any suspicion of leukemia. Indeed, such is the recommendation in many text-books. However, the statistical error inherent in random sampling is very high if only 100 cells are counted. This is due to the irregular distribution of the cells and is readily verified by counting the eosinophils in several groups of 100 cells in different areas of the same slide or in different slides from the same sample of blood.[2] The extent of the statistical error is readily determined from charts of confidence belts.[1,2] In the case of 5% eosinophils in a 100 cell count, we may be nearly certain (95% certain, i.e., confidence or fiducial coefficient of 0.95) that the true value lies between 2% and 12%. Such a broad range covers normal, borderline and definitely elevated levels and indicates the magnitude of the possible error. We are in agreement with Goldner and Mann[2] that, ". . . other errors, which are truly experimental could be kept sufficiently constant if a standard technique was adopted so that the only error with which one need be concerned was the statistical one."

Method

The statistical error is greatly decreased by counting larger numbers of cells. The 95% confidence limits for 5% eosinophils in a count of 100 cells are 2% and 12% while these limits for a count of 250 cells are 3% and 8.5%, and for a count of 1,000 cells are 4% and 6.5%. In order to take advantage of this increase in acuracy we have resorted to examining 500 cells for eosinophils only, whenever the eosinophil percentage is above 5 in an otherwise normal routine 100 cell differential count. By the simple expedients of mounting the blood smear with a large cover slip and doing the 500 cell count under the high dry objective, the procedure is no more time consuming than an ordinary 100 cell differential carried out under the oil immersion lens. Eosinophils are readily identified under the high dry objective, and no attempt is made to identify any other cell types. The differential of the routine 100 cell count is reported with a note concerning the corrected eosinophil percentage.

* Received for publication August, 1957.

In order to permit statistical evaluation of the method, a single observer performed sixty-seven 100 cell differential counts, and several days later performed 500 cell eosinophil counts on the same slides, without reference to the original results.

Results

The 67 slides studied included 35 with normal eosinophil levels Group A) and 32 with slightly or moderately elevated eosinophil levels (Group B) based on the routine 100 cell differential count. Although the textbooks record the upper limit of the normal adult eosinophil count as 3 or 5%, or 250, 300, or 400 cells per cmm., we considered a count abnormal only if it exceeded 5% or 400 cells per cmm. In our experience most clinicians consider 5% a borderline value, so we preferred to include it with the normals. In most cases evaluation on the basis of percentage was the same as evaluation on the basis of absolute numbers, but there were a few exceptions. A count was considered elevated if either method of evaluation gave high values.

Reclassification of the slides by 500 cell counts according to our method yielded 48 normal and only 19 elevated eosinophil levels. Therefore thirteen cases thought to have eosinophilia in the routine count were found normal in the 500 cell count. In no case was the classification changed from normal to elevated by the 500 cell count. The distribution of the results is presented in Table 1.

TABLE 1
DISTRIBUTION OF CASES ACCORDING TO EOSINOPHIL LEVELS

	NORMAL	ELEVATED		
	5% or Less 400 Cells or Less	More Than 5% 400 Cells or Less	5% or Less More Than 400 Cells	More Than 5% More Than 400 Cells
100 cell count . .	35	0	2	30
500 cell count . .	48	2	4	13

These changes in classification indicated that results based on routine 100 cell counts tend to be too high. The average eosinophil counts by the routine 100 cell count were 2.37% for Group A slides and 8.37% for Group B slides, while the averages by the 500 cell count were reduced to 1.97% for Group A slides and 5.23% for Group B slides. Of the 35 slides in Group A, the 500 cell eosinophil counts were lower than the routine 100 cell counts in 21 instances, higher in 9 instances and unchanged in 5 instances. All of the 32 slides in Group B were lower in the 500 cell eosinophil count than in the routine 100 cell count. These results indicate not only that the eosinophil percentages reported in routine counts tend to be high, but also that the inaccuracies are greater in cases of apparent eosinophilia than in apparent normals.

It was necessary to apply statistical methods to prove that the generally lower values obtained by 500 cell counts were statistically significant. The observations were paired and the differences recorded, the standard deviation of the differences between pairs was calculated, and the T-test applied. The results indicated that the differences obtained in Group A were valid at the 5% level of significance (i.e., $\alpha = 5\%$) but

not at the 2.5% level of significance. The differences in Group B were larger so that the results were valid even beyond the 0.5% level of significance.

Discussion

The statistical error in eosinophil enumeration in routine differential counts is very high due to irregular distribution of the cells. The statistical error is greatly diminished by counting 500 cells using the technique described and it is suggested that this be done whenever the routine 100 cell count yields an eosinophil percentage above 5. The results show that the more accurate counts tend to be lower, and this was verified by statistical analysis. Actually, this result could be anticipated because it is based on the assymetrical shape of the statistical distribution curve for counts other than 50%.[2] The results emphasize the practical importance of the problem, because it is the high eosinophil values that are taken to have clinical significance. Many supposedly high counts will become normal if more cells are counted in order to overcome errors of distribution.

The technique presented is not intended as a substitute for chamber methods for counting absolute numbers of eosinophils. The purpose of our method is to reduce purely statistical inaccuracies that result in false reports of eosinophilia in routine differential counts.

Summary

Eosinophil levels determined from routine 100 cell differential leukocyte counts are subject to considerable error due to irregular distribution of the cells on the slide. The error is in the direction of giving incorrect high values. In all cases of eosinophil percentage above 5 in otherwise normal routine differential counts, it is suggested that eosinophils alone be enumerated among 500 cells on a mounted slide under the high-dry objective. This procedure increases the accuracy of the eosinophil percentage and usually gives lower values. The corrected values are frequently in the normal range, thus eliminating unnecessary laboratory procedures which frequently follow incorrect reports of eosinophilia.

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ACID MYCOPHIL AGAR FOR THE SELECTIVE ISOLATION OF YEASTS*

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During the course of a study to determine the efficiency of neomycin and terramycin combined separately with sulfathalidine to reduce the microbial flora of the intestinal tract of humans prior to surgery (1), difficulty in obtaining quantitative counts of yeasts using Littman medium (BBL), or Littman oxgall agar (Difco), with streptomycin was encountered. This inability to obtain some counts for yeasts was due to the disproportionate number of bacterial cells resistant to any one or all of the bacterial inhibitors, viz., gentian violet, oxgall, and streptomycin. The resistant bacteria overgrowing the yeasts in the lower dilutions of fecal material belonged to three genera: *Streptococcus, Aerobacter,* and *Pseudomonas.* In an effort to eliminate this interference by the resistant bacteria, several media designed to inhibit bacteria without interfering with the yeasts were tested (Table 1). Mycosel agar** (BBL) inhibited strains of *Streptococcus* and *Aerobacter* but not *Pseudo-*

TABLE 1

Growth of Certain Fungi and Bacteria on Media Selective for Fungi

Organisms	Mycosel	Mycophil pH 4	Littman
	Observations at 6 days		
Microsporum audouini	+	+	+
M. gypseum	+	+	+
Trichophyton mentagrophytes	+	+	+
T. rubrum	+	+	+
Sporotrichum schenckii	+	+	+
Monosporium apiospermum	+	+	+
Hormodendrum pedrosoi	+	+	±
Phialophora verrucosa	+	+	±
Nocardia brasiliensis	—	—	—
N. asteroides	—	—	—
Streptomyces griseus	+	+	+
Blastomyces dermatitidis	+	—	+
Histoplasma capsulatum	+	—	±
	Observations at 4 days		
Aspergillus sp.	+*	+	+
Penicillium sp.	+*	+	+
Mucor sp.	+*	+	+
Neurospora sp.	—	+	+
Cryptococcus sp.	+	+	+
C. neoformans	±	+	+
Candida albicans	+	+	+
C. parakrusei	—	+	+
Geotrichum candidum	+	+	+
Staphylococcus aureus	—	—	—
Streptococcus faecalis	—	—	+
Strep. viridans	—	—	—
Pseudomonas aeruginosa	+	—	+
Pseudomonas sp.	+	—	+
Escherichia coli	—	—	—
Proteus vulgaris	—	—	—
Aerobacter sp.	—	—	+

\+ = growth
— = no growth
± = doubtful growth
* Limited growth

monas. Mycophil agar (BBL), with the pH of the medium reduced to pH 4-4.5 by the addition of 0.3 ml of 10% lactic acid per 20 ml of medium (2), inhibited the greatest majority of the bacteria encountered under the circumstances. This medium (Acid Mycophil agar) was used in numerous samplings of fecal specimens and was found to be highly effective in allowing the quantitative enumeration of *Candida* even when the yeast counts were as low as 10^2 and the bacterial counts as high as 10^9 per gram of wet feces. In the absence of resistant bacteria, simultaneous counts with Acid Mycophil agar and Littman medium with streptomycin were comparable (Table 2).

TABLE 2

Comparison of Plate Counts for Yeast in Feces Using Littman Medium and Acid Mycophil Agar

Specimen No.	Plate counts per unit wt. or vol of fecal specimen	
	Littmans	Mycophil pH 4
22–2	46×10^3	35×10^3
31–2	37×10^2	35×10^2
31–3	7×10^2	4×10^2
33–1	1×10^2	3×10^2
39–1	$<10^2$	$<10^2$
39–3	1×10^2	2×10^2

Although the colonial morphology of the filamentous fungi on Acid Mycophil agar is not typical of that seen on Sabouraud glucose agar, the morphology of the yeasts is unaltered. This medium seems quite effective in inhibiting bacteria and is recommended for use in the isolation of yeasts and of most pathogenic filamentous fungi in circumstances where bacteria greatly outnumbers the fungi. The low pH, however, does inhibit some pathogenic fungi, e.g., *Nocardia*, *Blastomyces*, *Histoplasma* (Table 1). Therefore, when one is attempting to isolate these organisms from clinical material, difficulties still will be encountered with Acid Mycophil agar as well as with other selective media for fungus isolations.

* Received for publication February, 1958.

** Contains Chloramphenicol and Actidione.

REFERENCES

1. Leo, W. A., von Riesen, V. L., Roberts, G. G., and Schloerb, P. R. 1958. Twenty four hour preparation of the large bowel for surgery using Neomycin-Sulfathalidine or Neomycin-Oxytetracycline: A comparative evaluation. Accepted for publication.
2. BBL Products 1956 *Products for the Microbiological Laboratory* Baltimore Biological Laboratories, Baltimore, Md.

AMONG THE NEW BOOKS

Many new volumes of interest to the medical technologist have appeared during the last quarter of 1957 and in 1958. As lazy as most of us Americans are about learning other languages beside our own, we're pleased to have a textbook published in 1950, brought to our attention. In this "age of the sputniks," a "Textbook for classes and self-study" —written "by a scientist for scientists" is

SCIENTIFIC RUSSIAN. By James W. Perry, a chemical engineer by training. Associate Professor, Modern Language Department, Massachusetts Institute of Technology, Cambridge, Mass. Interscience Publishers, Inc., New York 1, N. Y. 1950. 816 pp. (including 127 pages of Russian English Vocabulary). $7.50.

This book is not suggested as "easy reading," but some of your "spare time" may be put to really worthwhile use.

Those of us who read *Eleven Blue Men* and other stories by Berton Roueché from *The New Yorker* a few years ago, are delighted to have his latest collection:

THE INCURABLE WOUND and Further Narratives of Medical Detection. Little, Brown & Company, Boston and Toronto. 1957. 177 pp. Six short stories. $3.50.

CLINICAL PATHOLOGY DATA. Second Edition. By C. J. Dickinson, B.A., B.Sc., B.M., M.R.C.P., Medical Registrar, Middlesex Hospital. (Late Resident Medical Officer, University College Hospital, Captain, R.A.M.C.). Charles C. Thomas, Publisher, Springfield, Illinois. 1957. 91 pp. $4.50.

Brief notes in table form give contents, normal values, and conditions for increase or decrease in the physical properties for which tests are made on various body fluids. Other tables include specific groups of tests with their interpretations. Still more have a listing of tests, diseases, "what tested for," methods used, and interpretation. This volume is most valuable for quick reference although most of the same information can be found through a more intensive search of other sources.

HOST-PARASITE RELATIONSHIPS IN LIVING CELLS, a Symposium sponsored by the James W. McLaughlin Fellowship Program, University of Texas Medical Branch, Galveston, Texas, April 27, 1956. Compiled and edited by Harriet M. Felton, M.D., Charles C. Thomas, Publisher, Springfield, Illinois. 1957. 245 pp. 28 illustrations, 2 tables, 3 color plates. $6.50.

More is given on the electron microscope and its role in present day cytological studies, followed by the subject of Cytopathology. Further studies on the Current Concepts of Host-Parasite relationships, including those in the field of Virology, Metabolic Relationships, and Parasite resistance make this contribution by an outstanding group of investigators one of considerable value to the cytologist.

LABORATORY APPLICATIONS IN CLINICAL PEDIATRICS. By Irving J. Wolman, M.D., Director of Laboratories and Hematologist, The Children's Hospital of Philadelphia; Associate Professor of Pediatrics, Undergraduate and Graduate Schools of Medicine, University of Pennsylvania; Editor, Quarterly Review of Pediatrics. The Blakiston Division, McGraw-Hill Book Company, Inc., New York, Toronto, London. 1957. 1019 pp. 72 Tables. Classified Bibliographies for each chapter. $15.00.

Pediatrics from the laboratory standpoint—or the laboratory from the point of view of the pediatrician. No matter how you express it, wherever these two medical specialties are in conjunction, this book will be in daily use in the laboratory. Written primarily for the physician, the medical technologist who works with children will find valuable references. Techniques are not included as such.

BLOOD GROUP SEROLOGY. By Kathleen E. Boorman, Senior Scientific Officer, South London Blood Transfusion Center, Sutton, Surrey, and Barbara E. Dodd, M.Sc. (Lond.), Ph.D. (Lond.), Lecturer in Forensic Medicine, The London Hospital Medical College. Little, Brown & Company, Boston, 1957. 317 pp. 30 illustrations, 43 tables. Appendices: I. A Practical Guide to Laboratory Procedure. II. Glossary. III. Numerical Index of Techniques. $7.50.

Techniques and practical applications of those techniques make this book one for the laboratory and blood bank library—simplification is the keynote.

A PRACTICAL MANUAL OF MEDICAL AND BIOLOGICAL STAINING TECHNIQUES. Second Edition. By Edward Gurr, F.R.I.C., F.R.M.S., F.L.S., M.I. Biol. Interscience Publishers, Inc., New York and London. 1956. 451 pp. $4.00.

Practical and useful—in any laboratory. Beside the older and better known stains and staining methods, the author has devoted sections to cytological methods, fluorescence microscopy, and histochemical procedures.

FUNDAMENTALS OF IMMUNOLOGY. Third Edition. By William C. Boyd, Professor of Immunochemistry, Boston University, School of Medicine. Interscience Publishers, Inc., New York and London, 1956. 776 pp. 69 illustrations, 94 tables. $10.00.

This edition is completely revised and rewritten. The contents provide background for the medical technologist and for the student of medical technology. Chapter 15 is devoted entirely to immunological techniques for the clinical laboratory.

AN ATLAS OF HUMAN HISTOLOGY. By Mariano S. H. Di Fiore, Associate Professor of Histology and Embryology, Faculty of Medical Sciences, University of Buenos Aires and Head of the Laboratory of the Juan A. Fernandez Hospital. Lea & Febiger, Philadelphia, Penna. 1957, 215 pp. 99 original color plates. 156 figures. $7.50.

This is designed to supplement the usual textbook of histology, but the sections and staining will be an incentive to every histology technician who studies them. The stains employed are named but techniques are not given.

AN ATLAS OF FETAL AND NEONATAL HISTOLOGY. By Marie A. Valdés-Napena, B.S., M.D., Associate Professor of Pathology, Women's Medical College of Pennsylvania, Assistant Chief in Pathology, Dept. of Laboratories, Philadelphia General Hospital, Assistant Medical Examiner, Dept. of Public Health, City of Philadelphia. J. B. Lippincott Company, Philadelphia and Montreal. 1957. 200 pp. 238 microphotographs. $11.00.

No color photography but the pictures are clear. Histology technicians will find less in this than in most of the other atlases of similar type—more for the department of anatomy.

HISTOLOGY. Third Edition. By Arthur Worth Ham, M.B., F.R.S.C., Professor of Anatomy, in Charge of Histology, in the Faculties of Medicine and Dentistry, University of Toronto; Head of the Division of Biological Research, Ontario Cancer Institute, Ontario, Canada. J. B. Lippincott Company, Philadelphia and Montreal. 1957. 894 pp. 582 illustrations, 8 color plates. $11.00.

This textbook of histology should be in every histology teaching laboratory. In addition to sections on the tissues and histology of the various systems, Part One is devoted to the study of histological techniques.

TEXTBOOK OF VIROLOGY for Students and Practitioners of Medicine. Third Edition. By A. J. Rhodes, M.D., F.R.C.P. (Edin.), F.R.S.C., Director, Sch. of Hygiene and Professor of Microbiology, Sch. of Hygiene, University of Toronto; Virologist, The Hospital for Sick Children, Toronto, and C. E. van Rooyen, M.D., D.Sc. (Edin.), M.R.C.P. (Lond.), F.R.C.P. (C), Professor of Bacteriology, Dalhousie Univ., Halifax, Nova Scotia and the Victoria General Hospital, Associate Dir., Nova Scotia Public Health Laboratory, Honorary Consultant in Virus Diseases, Royal Canadian Naval Hospital, Halifax. The Williams & Wilkins Company, Baltimore. 1958. 642 pp. 81 illustrations. 22 tables. $10.00.

This edition has necessarily had considerable revision as the result of recent advances in virology. The authors state in their Preface that their objectives, namely, "to present an account of the essential features of the virus and rickettsial diseases of man, in a form suitable for graduate students of medicine, bacteriology, virology, and public health," remain unchanged, but in addition, they hope that the attention given to laboratory diagnosis "will assist physicians who have access to such facilities."

AIDS TO BACTERIOLOGY. Ninth Edition. By H. W. Scott-Wilson, B.Sc., B.M., B.Ch. (Oxon.), Director of the Laboratories of Pathology and Public Health, London. The Williams & Wilkins Company, Baltimore. 1957. 403 pp. Appendix Table of Identification. $3.50.

A handbook of Microbiological identification that is primarily for public health laboratories. The nomenclature is that of Bergey's Manual except in such cases that this is not commonly used in England.

BERGEY'S MANUAL OF DETERMINATIVE BACTERIOLOGY. Seventh Edition. By Robert S. Breed, Late Professor Emeritus, Cornell University, Geneva, New York. E. G. D. Murray, Research Professor, University of Western Ontario, London, Ontario, Canada. Nathan R. Smith, Senior Bacteriologist, Retired, Plant Industry Station, U. S. Dept. of Agriculture, Beltsville, Md., and 94 Contributors. The Williams & Wilkins Company, Baltimore. 1957. 1094 pp. $15.00.

A new edition of the generally accepted authority for bacteriological nomenclature has eliminated inadequately described organisms as well as those which are difficult to place. This volume will continue to be the authority for medical writing in its field.

MEDICAL WRITING. Third Edition. By Morris Fishbein, M.D., formerly Editor, *The Journal of The American Medical Association*, Contributing Editor, *Postgraduate Medicine*, Editor, *Excerpta Medica*, Amsterdam. The Blakiston Division, McGraw-Hill Book Company, New York, Toronto, and London. 1957. 262 pp. 36 illustrations. $7.00.

Medical technologists preparing to submit papers for publication, awards or convention programs would do well to purchase a copy of this volume, and to study it carefully before and while revising such articles.

CLINICAL PARASITOLOGY. Sixth Edition. By Ernest Carroll Faust, A.B., M.A., Ph.D., The William Vincent Professor of Tropical Diseases and Hygiene, Dept. of Tropical Medicine and Public Health, Tulane Univ. School of Medicine, New Orleans, La.; Consultant, U. S. Public Health Service; Member, Expert Panel on Parasitic Diseases, World Health Organization; Member, Committee on Revision, U. S. Pharmacopeia, 1951-1960; Field Coordinator, Tulane-Colombia Program in Medical Education, U. S. Operational Mission, International Cooperation Administration; and Paul Farr Russell, M.D., M.P.H., Staff Member, the Rockefeller Foundation; Consultant to Surgeon General, U. S. Army; Member Malaria Panel and formerly Chairman, Expert Committee on Malaria, World Health Organization; Formerly Malaria Consultant to Tennessee Valley Authority and to the U. S. Public Health Service; with the Editorial Assistance of David Richard Lincicome, B.S., M.S., Ph.D., Howard University, Washington, D. C.; Editor of the *Journal of Experimental Parasitology*; Guest Scientist, Dept. of Parasitology, Naval Medical Research Institute, National Naval Medical Center, Bethesda, Md.; Lieutenant-Colonel, Medical Service Corps, Army of the United States. Lea & Febiger, Philadelphia. 1957. 1078 pp. 346 illustrations, 7 colored plates, 22 tables. $15.00.

Aside from the generally revised sections in view of advanced knowledge of diseases caused by animal parasites, the medical technologist will be particularly

interested in Section V, the Technical Appendix, which gives detailed techniques for the examination of specimens for parasites. The balance of the book will be invaluable, just as former editions were, to the student, as well as to the medical technologist in the clinical laboratory.

ATOMIC ENERGY IN MEDICINE. By K. E. Halnan, M.D., D.M.R.T., London. Philosophical Library, Inc. New York. 1957. 157 pp. 31 illustrations and charts. 11 tables. 14 plates. $6.00.

One of the "Atoms for Peace" series, this volume gives some of the more important applications of atomic energy in the field of medicine. It is easy to read, not for techniques, but for general information.

An array of new books on various phases of Biochemistry has appeared these past few months.

TRACE ANALYSIS. Papers presented at a Symposium on Trace Analysis held at the New York Academy of Medicine, New York, N. Y., Nov. 2, 3, & 4, 1955. Edited by John H. Yoe, M.S., M.A., Ph.D., Professor of Chemistry and Director, John Lee Pratt Trace Analysis Laboratory, Univ. of Virginia, and Henry J. Koch, Jr., A.B., M.D., Sloan-Kettering Institute for Cancer Research, New York, N. Y. John Wiley & Sons, Inc., New York. 1957. 672 pp. 192 illustrations. 72 tables. $12.00.

A collection of 24 papers by authorities in their respective fields, which gives the various applications of trace analysis. The phases discussed range from Chemical Microscopy through Flame Spectrometry, Gamma-Ray and Mass Spectroscopy to Microbiological Techniques. There is also a section on Instrumentation. While this is not currently of prime interest to the medical technologist in the clinical laboratory, the research angle will have appeal.

QUANTITATIVE PHARMACEUTICAL CHEMISTRY. Fifth Edition. By Glenn L. Jenkins, Ph.D., Professor of Pharmaceutical Chemistry and Dean, John E. Christian, Professor of Pharmaceutical Chemistry, both of Purdue University School of Pharmacy, and George P. Hager, Ph.D., Senior Scientist, Smith, Kline & French Laboratories, Blakiston Division, McGraw-Hill Book Company, Inc., New York, Toronto, London. 1957. 552 pp. 61 illustrations. 80 tables. $7.00 or $8.50.

Of particular value to the medical technologist with its applications of quantitative analysis, this book is primarily written for the pharmacy student. Techniques are given in detail.

METHODS OF BIOCHEMICAL ANALYSIS. Volume IV. Edited by David Glick, Professor of Biological Chemistry, University of Minnesota. Interscience Publishers, Inc. New York & London. 1957. 362 pp. 46 illustrations. 29 tables. $8.50.

This entire series is of much practical value to the clinical chemistry laboratory. Volume IV has an impressive group of contributors, both from the U. S. and abroad. Many of the techniques and methods are given in detail.

METHODS OF BIOCHEMICAL ANALYSIS. Volume V. Edited by David Glick, Professor of Biological Chemistry, University of Minnesota. Interscience Publishers, Inc. New York & London. 1957. 502 pp. 40 illustrations. 43 tables. $9.50.

Volume V will be of even more practical value than the other four, with its sections on Estrogens, Vitamins, and Hormones, as well as those on trace analyses.

COLORIMETRIC ANALYSIS. Volume I. Second Edition. By Noel L. Allport, F.R.I.C. and J. W. Keyser, M.Sc., Ph.D. (Lond.), A.R.I.C. The Macmillan Company. New York, N. Y. 1957. 424 pp. 11 illustrations. 21 tables. $9.00.

Detailed methods are given, using colorimetric techniques for various determinations in urine, blood, tissues, spinal fluid, etc. Some of these are adaptations for more than one medium. The usual section gives a brief discussion of available methods for a specific determination, then gives each method with a discussion specifically related to it. This would be an asset to the laboratory library.

EXPERIMENTS IN BIOCHEMICAL RESEARCH TECHNIQUES. By Robert W. Cowgill, Ph.D., Department of Biochemistry, University of Colorado School of Medicine, and Arthur B. Pardee, Ph.D., Department of Biochemistry and Virus Laboratory, University of California. John Wiley & Sons, Inc., N. Y., and Chapman & Hall, Ltd., London. 1957. 189 pp. 21 illustrations. 16 tables. $3.50.

This book contains a series of experiments giving the objectives, principles, equipment and supplies required, procedure, treatment of data, questions and references for each. The biochemistry of enzymes and radioactive isotope tracer techniques are the main subjects treated. Section I gives the physical chemical methods for the separation and identification of biologically important compounds.

CHEMICAL METHODS IN CLINICAL MEDICINE. Fourth Edition. G. A. Harrison, B.A., M.D., B.Ch. (Cantab.), M.R.C.S. (Eng.), L.R.C.P. (Lond.), F.R.I.C., Consultant Pathologist, South West Metropolitan Regional Hospital Board at St. Richard's, the Royal West Sussex and Graylingwell Hospitals, Chichester, Sussex. Grune & Stratton. New York. 1957. 667 pp. 5 color plates. 158 illustrations. Tables. $11.00.

The methods of chromatography and electrophoresis are added in this volume. Apparatus required for these tests is detailed in the first chapter. The entire book seems rather more detailed than most, especially with the material given on the clinical application of data.

PRACTICAL CLINICAL BIOCHEMISTRY. By Harold Varley, M.Sc., F.R.I.C., Biochemist, Manchester Royal Infirmary, Lecturer in Clinical Pathology, Manchester University. Interscience Publishers, Inc. New York, N. Y. London. 1954. 551 pp. 70 illustrations. 31 tables. $6.50.

Another good, PRACTICAL volume for the hospital chemistry laboratory. Besides detailed techniques, there is a section on the interpretation of each test.

PRACTICAL CLINICAL CHEMISTRY. Second Edition. By Alma Hiller, Ph.D., Associate Attending Biochemist in Charge of Clinical Chemistry, Presbyterian Hospital, Chicago and Associate Professor of Biological Chemistry, University of Illinois College of Medicine. Charles T. Thomas, Publisher. Springfield, Illinois. 1957. 265 pp. 18 tables. $6.50.

Featured in this edition are simplified procedures. These are especially adapted to use in the routine clinical laboratory. Each determination is described in detail, giving a list of any special apparatus needed, as well as methods for preparing reagents, procedures, and calculation of results.

MICROTECHNIQUES OF CLINICAL CHEMISTRY. For the Routine Laboratory. By Samuel Natelson, Sc.M., Ph.D., Department of Biochemistry, Rockford Memorial Hospital, Rockford, Illinois. Charles T. Thomas, Publishers. Springfield, Illinois. 1957. 484 pp. 152 illustrations. 11 tables. $11.00.

With an ever increasing interest in the use of microtechniques, not only in pediatrics, but in the average clinical chemistry laboratory, this volume should give the solution to many problems. It may be pointed out that Dr. Natelson was a guest speaker at the Silver Anniversary convention of ASMT in Chicago in 1957. The chapter on "Principles Commonly Used in Microchemistry" points out that microanalysis "does not usually require special equipment not normally found in the clinical laboratory." Routine determinations are detailed for more than 75 constituents of the blood. The appendix contains much useful material that would apply to macrotechniques as well as micro.

THE CHEMISTRY OF BLOOD COAGULATION. Paul Morawitz. Translated by Robert Hartmann, M.D. and Paul F. Guenther, M.A., Ph.D., from the Departments of Medicine and German of Vanderbilt University, Nashville, Tennessee. Charles T. Thomas, Publishers. Springfield, Illinois. 1957. 194 pp. 2 tables. 2 illustrations. $4.50.

This volume will be of value as background material for the more recent advances in the field of blood coagulation. The translation of the monograph is accompanied by a glossary that explains that part of the terminology now obsolete. The 42 pages of Bibliography will give a picture of the theories on coagulation that were developed in the late 19th and early 20th centuries.

THE CHEMISTRY OF ORGANIC MEDICINAL PRODUCTS. Fourth Edition. By Glenn L. Jenkins, Professor of Pharmaceutical Chemistry and Dean of the School of Pharmacy, Purdue University, Walter H. Hartung, Professor of Pharmaceutical Chemistry, Medical College of Virginia, Kenneth E. Hamlin, Jr., Assistant Director of Chemical Research, Abbott Laboratories, and John B. Data, Assoc. Professor of Pharmaceutical Chemistry, The School of Chemistry, Purdue University. John Wiley & Sons, Inc. New York. 1957. 569 pp. 41 tables. $10.75.

This volume of advanced organic chemistry will be of more value to the clinical chemist than to the average medical technologist or student.

DANGEROUS PROPERTIES OF INDUSTRIAL MATERIALS. By N. Irving Sax, Consultant on Industrial Safety, Nuclear Development Corporation of America, White Plains, N. Y. Reinhold Publishing Corp. New York, N. Y. 1467 pp. $22.00.

Over 8500 materials are described, with details given in regard to composition, toxic hazard rating, fire hazard, explosion hazard, disaster control, ventilation control, storage and handling. Sections on Ventilation Control, Toxicology, Radiation Hazards, Industrial Fire Protection are given in addition to the specific hazards for each compound.

HANDBOOK OF TOXICOLOGY. Volume II Antibiotics. By the Committee on the Handbook of Biological Data, Division of Biology and Agriculture, the National Academy of Sciences, The National Research Council. Wright Air Development Center, Air Research and Development Command, United States Air Force, Wright-Patterson Air Force Base, Ohio, May, 1957. 264 pp. 1[illegible] references.

For these references such details are given as the source, molecular formula and weight, melting point, solubility, stability, biological activity in vitro, toxicity, etc.

A GROUP OF PAPERS ON MEDICAL WRITING. Second Printing. Published by Parke, Davis & Company.

Other Books Received:

PHYSICAL METHODS IN PHYSIOLOGY. By W. T. Catton, M.Sc., Physiology Dept. King's College, Newcastle-upon-Tyne, England. Philosophical Library. New York. 1957. 375 pp. 156 illustrations. 11 plates. 7 tables. $10.00.

This will be of little practical value to the medical technologist except as a reference book, in that it treats primarily of the application of physical experimental techniques applied to physiological investigation.

THE CEREBRAL CORTEX AND THE INTERNAL ORGANS. By Konstantin M. Bykov, M.D., translated and edited by W. Horsley Gantt, M.D., Collaborator in Pavlov's Laboratories 1926-29; Director of Pavlovian Laboratory and Associate Professor of Psychiatry, Johns Hopkins University. Chemical Publishing Company, Inc. New York. N. Y. 1957. 448 pp. 213 illustrations. $15.00.

NEW UNGUENT BASES AND LOTIONS. By I. K. Hoffman, B.S., Pharm. D., Consulting Chemist, Research Staff, U.C.L.A. School of Medicine. Chemical Publishing Company. New York. 1957. 160 pp. $4.75.

PSYCHOPATHIC PERSONALITIES. By Harold Palmer, M.D. Philosophical Library. New York. N. Y. 1957. 179 pp. $4.75.

THE CHRONICALLY ILL. By Joseph Fox, Ph.D. Philosophical Library. New York. N. Y. 1957. 229 pp. $3.95.